

Defining half a global problem

The World Commission on Environment and Development has had its heart in the right place but its head has been in the sand (or even in the clouds).

ANY organization called the *World Commission on Environment and Development* is almost destined to be platitudinous. Gro Harlem Brundtland, the prime minister of Norway, has done her level best to avoid that trap. Her most obvious problem, during the four years of her commission's existence at the instance of the UN General Assembly, was to reconcile the spirit of the keywords in her commission's name — "world", "environment" and "development".

On the evidence of her commission's report, published last week (*"Our Common Future"*, OUP, Oxford and New York; 1987), she has not succeeded. Worse than that, indeed, after prefacing her report with a reference to the "several years of political struggle, nationally and internationally" in which she has been engaged, she lends her name to a superbly readable but otherwise not particularly original statement of the global ecological problem, well larded with statements to the effect that it would all be different (and much easier) if people's sense of commonality were greater. The odd gap in the logic of the commission's report is that chauvinism and its dual (xenophobia) are recognized as the chief impediments to ecological civility, but that most of its recommendations bear on the environment and not on the manners of those who live in it. For one who, by her own admission, has struggled for so long, this is a strange omission.

The commission's canvass is familiar. Although the gross economic growth of the developing nations is respectable, it is patchy and, more to the point, usually slower than the growth of population, with the result that prosperity per head declines. Moreover, even this modest growth coupled with the voracious demands of the rich countries for natural resources places unprecedented demands on the surface of the Earth. The commission argues for "sustainable development", that which "meets the needs of the present without compromising the ability of future generations to meet their own needs". Among other things, this requires "a change in the content of growth" to make it less dependent on material resources and energy "and more equitable in its impact".

Eloquent

The report continues with sensible and even eloquent discussions of the several aspects of these problems — global environmental threats such as the greenhouse effect, regional problems such as those in rapidly changing river basins and the general insecurity caused by fluctuations of food supply in various parts of the world. The commission notes the several attempts that have been made, by fisheries agreements and even the Law of the Sea, to regulate the usually inimical interaction between exploitation in the interests of development and the integrity of the environment. It goes on to ask that there should be more deliberate mechanisms for identifying emergent problems, for the working out of their solution and even for the international enforcement of the legal instruments judged essential.

Recognizing that much of what it wants to see happen will be attainable only if there are substantially greater funds at the disposal of international agencies, the commission takes a leaf out of the book of the Brandt Commission which, six years ago,

advocated schemes for international fund-raising by means of taxes on such things as seabed minerals and the volume of international trade. It recognizes that all this will come about only if the "attitudes" of governments and their constituents can be "changed".

Would it not have been better to tell it like it is? Well-developed nation states that happen not to be super-rich are desparately but democratically worried about their future economic status relative to the very rich. Britain, most of Western Europe and even Norway are in that case. Japan is evidently different; the United States believes it is. And the plain truth is that none of these sovereign states believes that it has more than a nominal duty to share its unsatisfying wealth with the nations near the bottom of the pile. There is no government in the world (Sweden probably does best) that spends so much on foreign aid that, if its example were followed by others similarly placed, the poor countries would be enabled to catch up.

Poverty

So it is inevitable that the poor become relatively poorer, which means that their living conditions (or environment) deteriorate; the only saving grace is that some among them (India in the past two decades, for example) occasionally break out of this depressing mould and find a way of managing their affairs constructively. If in the process of becoming semi-rich, they think it necessary to cut down a few hundred hectares (or square kilometres) of primeval rain-forest, it will be found that local people are less regretful than Ms Brundtland.

To note these circumstances is not to admire them. In nation-states where people vote, selfish motives inevitably take precedence. It would be easier to persuade people that different systems of government would be preferable if it were not that the known alternatives are either inefficient or corrupt, and sometimes both. The World Commission's report is naturally full of pleas that there should be effective means of coordinating global enterprises, directed both to the management of the "global commons" and the enhancement of the prosperity of the disadvantaged, but it is curiously lukewarm in its advocacy of its own sponsor, the United Nations, as an effector of this coordination (although, in passing, there are some kind words for the specialized agencies). But if the essence of the conclusion of a consideration of our common future is that there must be created a mechanism for its better management, and if such a mechanism exists already, does it not rest with a World Commission either to applaud it or to say what may be wrong with it?

The problem that the commission should have tackled is, unfortunately, even more obdurate than the task it set itself. In the real world, the difficulty is that of avoiding catastrophe while governments, in what they consider the best interests of their people, are selfish, feckless and often both. The sheer breadth of the canvass on which the commission has conducted its enquiry is, in that connection, more of a distraction than a pointer to what needs doing. The most urgent need is not so much for yet another rehearsal of all the potential problems whose sheer weight might depress people into inaction, but for a pointed list of practical tasks that must be done.



Although everybody's list is different, avoidance of nuclear war is at the top of most lists, as it should be: there could hardly be a more effective way of setting back the cause of development. It is not especially helpful, except as part of a recipe for the ideal world, that the commission, having recognized this, should also rail at the waste of economic resources spent on the manufacture of armaments of all kinds. (Many of the states most in need of funds for civil development are paradoxically among the big spenders in this way.) The avoidance of a collapse of the international monetary system is another threat both to the environment and development, and might have been put higher on the commission's list. The likelihood that continued accumulation of carbon dioxide in the atmosphere will cause often-unwelcome climatic changes is another headache, not so much because the threat of drastic change is imminent, but because few people believe that existing international legal instruments could be adapted quickly to abate continued accumulation (but the attempt to tackle the problem of the ozone layer — see opposite — is for many people a welcome precedent). Helping to tell what weight should be given to these issues, and how, would have been a better use of the commission's time.

That does not imply that the other problems in the commission's long list should be put on the back burner for the time being, but merely that they may not deserve equal time and attention. Not even the preservation of endangered species? Sadly, the conservationists must be reconciled to losing many of the battles that lie ahead. A group of largely industrialized nations has reluctantly, some say unsatisfactorily, helped to conserve the whale population, whose commercial exploitation has become of marginal value, but is there a realistic hope that the trade in south-east Asian monkeys, a source of cash in an impoverished region, can be halted quickly, or in time to save some endangered species? And which is the more urgent need, that or the avoidance of nuclear war? Much the same may have to be said about many of the regional environmental problems listed by the commission. Development and the preservation of the environment are not necessarily inimical, and external donors can exercise a powerful influence on recipients' environmental policies. But what if the recipient governments do share the commission's views, or if they put survival first?

Even the issue of the survival of the poorest populations is not, in the real world, an absolute consideration. The classical case for generous overseas assistance — that grinding poverty, however isolated and distant, is a threat to international stability — seems to carry less weight each year with the rich countries of the world. The World Commission is right to wish that things were otherwise, but they are not, as yet. A small part of the explanation (not an excuse) is the disappointment of recent decades. While much aid has been misconceived, many recipients have been feckless in that role. It remains, for example, to be told to what extent the famines of recent years in Ethiopia are attributable to the policies of an ideological government? It must naturally be hoped that attitudes will change, but this is a general need not confined to the owners of the world's wealth.

It may be asking too much that the commission should have spelled out these unpalatable truths, but the consequence is that its report will not be easily distinguishable from other pronouncements on the same group of subjects. Nor will it, in a world not yet suffused with sweetness and light, help to concentrate attention on what needs most urgently to be done. That is a great misfortune, if only because the underlying issues are every bit as important as Ms Brundtland and her colleagues say. The commission sees its work being continued with support from the United Nations by means of a special programme and a series of conferences intended further to stimulate concern. The more urgent need is that a mechanism should be sought to determine what tasks should take priority in an imperfect world. As previously, the present generation may have to dump on its successors much of what it cannot accomplish by itself. With luck, succeeding generations may be better able to provide solutions.

Europe's research stall

The British Government should now be more constructive about Europe's research programme.

THE continuing dispute between Britain and the European Commission over Europe's planned research spending during the next 4.66 years (see page 5) is in danger of getting out of hand. The origins of the quarrel (in which, originally, West Germany sided with the British) is the belief that the present pattern of the commission's spending on research is inappropriate to the emerging need. The case is easily sustained. The relevance to practical needs of much of the applied research at the commission's joint research centres is not easily understood. Even some of the programmes in whose conception the commission thinks it has been forward-looking are ill-designed: the common programme of pre-competition research and development in information technology, for example, would have been more convincing over the past six years if participants had not been required by the commission's procedures to cobble together applications for support with indecent haste. But it will serve nobody's purpose if the British simply complain that they do not approve of what is planned. Should they not now say what they would wish to see instead?

Much of what the commission does in the name of research is admirable. This is eminently true of the thermonuclear research programme, represented for the time being by the machine called JET which is in many ways a telling model for what the commission should be doing. For this is a field whose possible importance is beyond dispute, and which no single member of the communities could follow effectively on its own. The commission's difficulty is that there are not many other fields of comparable importance and similar justification on which the commission might spend comparable funds. The development of fast reactors might have been a candidate research programme in the old days of Euratom's supremacy, but that horse has bolted (chiefly to France). Similarly, the development of space launchers and of the machines they might launch is in the hands of national agencies, private and public, as well as of the European Space Agency, whose membership is not identical with that of the communities.

The commission's underlying dilemma is familiar even to national governments seeking to use central funds for the sponsorship of economically valuable research. Basic projects are welcomed, but do not yield immediate benefit, but collaborative projects nearer the market are either already in the hands of companies, or are likely to be regarded with jealousy by the parties most concerned. Hitherto, the commission has sought to turn this difficulty by requiring that companies from different member states should jointly work (and invest their own funds) in projects in, for example, information technology. Too little effort has been spent on the assessment of this technique, but the anecdotal evidence is not encouraging. Instead of acting as dog in the manger, the British government could usefully specify tests that might satisfy its objections.

Meanwhile, there is plenty else the commission could attempt. By common consent, those who work as scientists in Europe are far less mobile than Europe needs them to be, but the commission's programme in this field is only modest. Then there are some fields of basic research in which central support, even on a modest scale, could help to enrich and strengthen European science and technology in unexpected ways. The British committee that reported last week on the reorganization of the earth sciences sensibly advocated a European initiative in ultra-high-pressure studies, for example. The commission's natural fear that such a step would merely be a sop to European academics would be misplaced. For, as in Britain in particular, so in Europe in the large, the range and richness of research is not nearly as great as economic, not merely academic, considerations dictate. □

Ozone layer protection deal still up in the air

- Targets for global chlorofluorocarbon use
- New results could alter timetable

London

A DIFFICULT bargaining session has led to a draft international agreement on reducing the global production and consumption of chlorofluorocarbons (CFCs). But the 31 countries taking part in last week's conference of the Vienna Convention on the Protection of the Ozone Layer in Geneva still have some important points to work out before a protocol can be signed this autumn.

The draft text for the agreement is dotted with phrases in brackets, indicating the items still to be discussed. Several points, however, have been agreed upon, including a freeze on the production and consumption of CFCs 11, 12, and 113 at 1986 levels by 1990, followed by a reduction of 20 per cent in 1992, with scientific evidence on the relationship between CFCs and ozone damage to be reviewed in 1990 and every four years after that.

Prospects for disagreement at the conference were apparent from the beginning — the United States, Canada, Scandinavian countries, New Zealand and Australia were pushing for the total phasing-out of all harmful CFCs, while the European Economic Community had agreed only to support a 20 per cent cut-back. Now, after four days of intensive negotiations, governments will be asked to choose between two proposals for a further 30 per cent reduction of CFC production, to be imposed either in 1994 with a simple majority vote of signatories, or in 1996 unless a two-thirds majority opposes it. Further reductions beyond that are still a possibility.

Other outstanding issues for discussion concern developing countries, who want to protect their use of CFCs as refrigerants. Proposals being considered call for developing countries to be exempt from the provisions of the convention for five years, or until their annual use of CFCs reaches 0.1 kg per head of population.

The final agreement, to be presented for signature in Montreal in September, appears likely to cover CFCs 114 and 115, as well as halons. Last week's meeting generally agreed on the need to regulate halons, thought to be one of the most highly damaging substances to the ozone layer, although the Soviet delegation pointed out that halons are not CFCs and therefore could not be legally covered by the conference. Dr Mostopha Tolba, executive director of the United Nations Environment Programme, is to seek a

mandate from the governing council allowing halons to be included.

Inclusion of CFC 113 in the restrictions was not part of the original position of the EEC delegation, and Japan had been particularly sensitive on the issue because the substance is used as a solvent in the microchip industries. The Japanese delegation appeared to soften, however, when the wording on the reduction of CFCs was modified to include "combined" adjusted annual production; they believe that reductions of the other CFCs would offset the need for cutting back on 113.

Major producing countries as well as Japan and the Soviet Union will meet again in Brussels at the end of June to tackle the remaining issues, following a meeting in May of the European environment ministers. The 31-nation working group will meet in Montreal just before the scheduled signing of the protocol.

Scientific evidence which may come to light before then, however, could change the calendar for the enactment of controls. The US National Ozone Expedition to Antarctica is scheduled for August. In addition, there could be scientific agreement on data submitted earlier this year to the House of Representatives which suggests much greater ozone depletion levels than originally predicted.

Kathy Johnstone

Rhyl not to blame



This picture from the OTA report on US marine waters prepared for Congress appears as an example of coastal waste discharge. But the photograph, supplied both to OTA and *Nature* by Greenpeace, was taken in Rhyl in North Wales, where officials say it is never used for sewage discharge. See "Coastal waters in jeopardy" on page 9.

Who decides US AIDS policy?

Washington

DISAGREEMENT within the US administration over how to control the spread of AIDS (acquired immune deficiency syndrome) have surfaced in a controversial speech by Education Secretary William Bennett, calling for the introduction of compulsory AIDS tests. Speaking at Georgetown University, Washington, last week, Bennett argued that there was a "good case" for making AIDS testing a "requirement" for hospital patients, potential immigrants, prison inmates and applicants for marriage licences. Even the principle of medical confidentiality may have to be reconsidered in light of the AIDS epidemic. Open debate was now needed, he said, to weigh the claims of "individual privacy and the well-being of other individuals, and the health of the public". He pointed to a growing line of cases indicating that doctors have a "duty to notify third parties of the risk of infection from a patient and that physicians may be held liable for breach of this duty".

Bennett's views, reminiscent of guidelines recently introduced in Japan (see page 8 in this issue), have not found favour with the Surgeon General, C. Everett Koop, and Public Health Service officials, who continue to believe that voluntary testing, offered in strict confidence, is the best way to find carriers of the AIDS virus. At a hearing of a House of Representatives subcommittee on health and the environment last week, Koop stressed that compulsory testing would lead to discrimination against AIDS-virus carriers and help drive the disease "underground".

Privately, officials of the Public Health Service are annoyed at Education Secretary Bennett's attempts to influence public health policy. Bennett had earlier criticized the content of planned AIDS education programmes and labelled emphasis on the use of condoms as "condom-mania" and "an evasion". A conservative, his support for "sexual abstinence among the young and sexual fidelity as the norm" as the best way to limit AIDS is echoed by President Ronald Reagan. But Bennett admits that his views are not shared by all members of the administration.

Given the disagreements, policy on compulsory testing and confidentiality now seems likely to be set by President Reagan. In response to criticism that the White House has failed to react quickly to the spread of AIDS — US spending on AIDS education is low compared to most European countries — and to increasingly pessimistic estimates of the scale of the epidemic, Reagan is believed to be preparing to set up a special commission on AIDS.

Alun Anderson

Britain's superconductor teams compare notes and budgets

London

THE first major meeting of British scientists working on high-temperature superconducting ceramics, held at the Rutherford Appleton Laboratory last week, was for several delegates a disappointment that failed to address important issues. The meeting, arranged by the Science and Engineering Research Council (SERC), aimed to identify "the strategy that should be followed by the UK for the new high-temperature superconductors". And it was well attended — originally, about 75 researchers closely involved in the field were invited, but the numbers eventually swelled to nearer 120. Many of those, though, had "muscle in", according to one prominent researcher, and in his view this resulted in time-wasting and detracted from the real business of the day.

The organizers hope that the meeting will result in greater collaboration between various groups, to avoid duplication and make best use of scarce funds. Talk of room-temperature superconductivity has now largely ceased, and the British groups are concentrating their efforts on the fundamental physics of the new ceramics. But chronic shortage of funds remains the major obstacle to Britain's chances of competing realistically with foreign countries, particularly the United States and Japan. "There is despondency," said one delegate. "Britain is behind — in the last three or four months we have just not had the resources."

At the last count, 12 groups had applied to SERC for grants in the next round totalling £1.4 million, out of SERC's physics committee's total budget for the round of £1.5 million. The committee's chairman, Professor Lawrie Challis, concedes that the situation is "very worrying".

In the short term there seems little hope of cash from other sources. Industrialists

were present at last week's meeting, but it was felt that the potential importance of the present work was not impressed upon them sufficiently strongly. The Department of Trade and Industry (DTI) sent a representative with a statement to assure delegates that DTI was aware of the importance of the field. However, he had no hard proposals for funding, and few dele-



The SERC Rutherford Appleton Laboratory, the venue for last week's British superconductor gathering, now has a new director, Dr Paul Williams. Dr Williams, aged 53, has been associated with the laboratory since 1960. The Rutherford Appleton's approach to the fundamental physics of superconductor ceramics is exemplified by the letter on page 45 of this issue.

gates held out much hope for the rapid cash support that is needed. A DTI spokesman in London said that the matter was being considered "at the highest level". The most likely source of DTI funding would come from its recently announced LINK initiative, which aims to encourage collaboration between universities and industry. But while research efforts are being concentrated on fundamental aspects, DTI's involvement remains doubtful in the near future.

Despite the misgivings, most delegates welcomed the opportunity to meet fellow workers. Greater collaboration might yet result, but the shortage of funds is stifling progress.

Simon Hadlington

Spending to keep up with the Japanese

Washington

IN tune with the popular theme of industrial competitiveness, the US National Science Foundation (NSF) has announced that special funds will be available for research into the new superconductors. The overt hope is that a solid foundation of research will enable US industries to gear up quickly for the anticipated commercial applications.

Three materials research laboratories, at Northwestern and Stanford Universities and at the University of Illinois at Urbana-

Champaign, will benefit from an immediate award of \$1 million. Another \$60,000 will be distributed as 'quick start' grants to researchers with promising techniques for turning the ceramic superconductors into usable forms and devices.

NSF director Erich Bloch admits the programme is an attempt to keep up with Japan, but says "American industry has also acted aggressively". He extols research into superconductivity as an example of the new-found cooperative spirit between universities and industry.

David Lindley

Transitions are hotting up

London

MEDIA coverage of "Soviet Science Day", celebrated each year in April, focused this year on the topical phenomenon of high-temperature superconductivity. According to *Pravda*, there are two teams working in this field in the Soviet Academy of Sciences, one at the Institute of Crystallography and one at the Lebedev Physics Institute. The latter team, headed by Dr Aleksandr Golovashkin, is working on yttrium ceramics and early in April reported superconductivity transitions commencing at 250 K in freshly-prepared specimens. With the passage of time, however, Golovashkin told *Pravda*, the transitions disappear.

According to the government news agency TASS, the team have also observed the Josephson effect in an yttrium ceramic at around 73 K, a result which, notes TASS, could be important for future computer technology.

Such announcements in the general media, in advance of scientific publication (due shortly in *JETP Letters*), are unusual. This departure is pre-sumably due partly to the current policy of *Glasnost* but also to a desire to be seen at the forefront of a field which is attracting such attention in the West. Golovashkin may have been over-modest in his interview however; according to unofficial reports at a recent meeting in Pisa, the Soviet transition is completed at 176 K, considerably higher than any other reported to date.

• David Lindley adds from Washington: Rumours of resistivity changes at 200 K or higher have circulated around US laboratories since the new ceramic superconductors were first discovered, but the measurements have been hard to reproduce, and full evidence for superconductivity has been lacking. J.T. Chen and colleagues, at Wayne State University in Detroit, claimed some weeks ago to have measured unambiguously the a.c. Josephson effect in ceramic samples, but the experiment is difficult and the signal can be mimicked by less exotic effects, such as formation of diodes at grain boundaries.

The Wayne State group sees a transition beginning at 240 K and completed by 200 K, and their result seems to be holding up. Presumably, grains of a different crystal structure superconduct at a higher temperature, but the whole sample does not become superconducting until cooled to 100 K because the grains are too sparsely distributed to allow a connected current path. Certainly, no one has yet come up with a bulk sample that is superconducting at 240 K and, as in the Soviet report, the high-temperature phase often disappears as ceramic samples age. □

Hybritech wins court injunction over sandwich assays

Washington

THE US biotechnology company Hybritech has scored yet another coup in defence of its much-contested patent for sandwich assays. A US judge is to issue a preliminary injunction forcing Abbott Laboratories to suspend sales of products using the sandwich assay technique.

Hybritech filed suit against Abbott last December, charging that some of Abbott's diagnostic tests infringed its patent for sandwich assays. The technique uses two monoclonal antibodies to make an antibody-antigen-antibody 'sandwich' which can detect small quantities of antigen for the early diagnosis of disease or pregnancy.

The preliminary injunction will affect several of Abbott's diagnostic tests for thyroid disorders, cancer and pregnancy. While these tests currently comprise only 7 per cent of Abbott's total diagnostic sales, if a permanent injunction results from the coming trial, it could freeze Abbott out of the potential \$5,000 million market for the diagnostic tests. The date for the trial has not yet been set.

The sandwich assay patent has been surrounded in dispute since Hybritech first sued Monoclonal Antibodies, Inc. for infringement in 1984. Hybritech lost the

case, and its patent, on the ruling that the technique was obvious to anyone skilled in the field. Hybritech appealed, and its patent was restored a year later under a more liberal interpretation of the evidence presented in Hybritech's laboratory notes to demonstrate its conception date.

Hybritech then re-entered its patent infringement suit against Monoclonal Antibodies, Inc. A preliminary injunction against Monoclonal Antibodies was announced a month ago, but has not yet been issued. Monoclonal Antibodies has more to lose than Abbott, because sandwich assays make up about 80 per cent of the company's product sales.

New evidence has come to light that may reopen the question of the validity of the Hybritech patent, however. The attorney for Abbott Laboratories, Robert Benson, has recruited a witness who claims to have discussed the sandwich assay technique at a conference in Seattle, Washington, in March 1979 — over a year before Hybritech applied for a patent. But the Hybritech conception date — the date from which the patent is active — was established in the previous trials as 4 January 1979. If this stands, Abbott's new witness may not be able to bail them out.

Carol Ezzell

Soft X-ray option tips balance towards Trieste synchrotron

Milan

IN spite of the continuing uncertainty about the next government of Italy, the plan to build a 1.5-GeV synchrotron radiation source has been given the go ahead, in the new research area around Trieste (see *Nature* 326, 324; 1987). To a large extent, the project owes its success to the enthusiasm of Nobel prize-winner Carlo Rubbia, now working at the European Organization for Nuclear Research in Geneva and teaching at Harvard University, who will have a lasting connection with the new machine. But the minister of research, Senator Luigi Granelli, has been an enthusiast for the project; his political effectiveness has not been dimmed by the political crisis.

Although Italy has a 15 per cent share in the European synchrotron at Grenoble, Rubbia says that there is a strong case for a national programme because of the need to operate with soft X rays, rather than with the hard X rays produced by the more powerful 5–10 GeV European machine. Rubbia notes that there is a similar trend towards lower energies both in the United States and in West Germany, embodied in

the machine called Bessy. In Rubbia's view, one objective of the Trieste machine will be to build up the pool of equipment and skill on the basis of which national groups can compete effectively in Europe.

Italy claims a long tradition in the use of synchrotron radiation, going back to the Adone machine built at Frascati, south of Rome, in 1967 by Bruno Touschek. The new 'third generation' machine at Trieste will keep Italy at the forefront of synchrotron technology, it is hoped. The project leader will be Massime Cornacchia, who is moving with a colleague from Berkeley, California.

Half of the cost of the new machine, which exceeds \$100 million, will be borne by the government of Italy and half by a consortium of the Region Friuli Venezia Giulia and the Trieste research area. The design has been supervised by a committee headed by Rubbia, and will be operated by a company called Sincrotrone Trieste. It is hoped that the feasibility study will be completed by the end of this year, and that construction will begin in 1988 with completion in 1992.

Paola De Paoli

European disunity

IN an effort to end the current deadlock over its £4,580 million high-technology research programme, the European Communities Commission is considering asking Britain to withdraw from all EEC research projects. Britain has been opposing the five-year research project, which is agreed by all other EEC governments, on the grounds that it is unfocused and poorly monitored. Unanimous agreement is essential if the programme is to go ahead, but if Britain decides not to participate the other 11 countries could proceed with what they see as an essential European response to US and Japanese high-tech research.

A formal request to Britain to withdraw voluntarily would be without precedent. But the British position has been strongly criticized by other EEC countries, and there have been warnings that some research teams may have to be disbanded if the stalemate is not resolved soon. British opposition to the research programme is thought to come partly from government departments; under Treasury rules, the cost of EEC research can be deducted from a ministry's budget.

K.J.

Shuttle stays earthbound

THE National Aeronautics and Space Administration (NASA) has abandoned plans to resume shuttle launches by 18 February 1988 following a decision by NASA to add two new tests before launching. NASA spokesman Jim Ball says the tests are not only important for the information they will give engineers about modifications to the shuttle, but will also give ground technicians a chance to try out new pre-launch procedures. NASA has made numerous modifications to the shuttle — some arising from investigations into the Challenger accident. Brakes, landing gear, main engines and the solid rocket motor have all been modified.

NASA estimates that there will be a 6 to 8 week delay to perform the fuelling and test firing tests. New launches are unlikely before the second half of next year.

J.P.

AIDS committee

THE Soviet Ministry of Health has proposed the establishment of an international coordinating committee for research on AIDS. Speaking on Moscow television, Dr Evgenii Chazov, the All-Union Health Minister, said the issue had already been raised with the World Health Organization (WHO), and that the Soviet delegation to the WHO General Assembly in May hopes to discuss it more fully. The proposed committee, Chazov said, would coordinate investigations into the nature of AIDS, diagnosis, the development of vaccines, and also preventative measures. International efforts would be important, Chazov said, in studying AIDS epidemiology.

V.R.

West German research agencies oppose new embryo law

Munich

THE two principal West German research organizations have issued long-awaited position papers in response to a proposed law which forbids research on human embryos. Both the Deutsche Forschungsgemeinschaft (DFG) and the Max-Planck-Gesellschaft (MPG) support the spirit of the law but would prefer that some experiments be allowed under the guidance of a committee of scientists, ethicists and theologians.

The papers came in response to a draft law first made public in April 1986, under which all research on human embryos and the genetic manipulation of human germ-line cells would be subject to criminal penalties, including imprisonment. The Justice Minister, Hans Engelhard (Free Democrat), is trying to push the new law through parliament by June.

Under the proposed law, procedures such as injuring or killing human embryos (except in the context of an abortion) are punishable by up to five years in prison. Cloning of embryos as well as creating chimaeras (hybrids) out of more than one

President Heinz Staab of the MPG said the proposed law "fixes conditions too strictly and does not allow for any flexibility." The MPG position paper goes further, stating that the introduction of criminal penalties will frighten scientists away from potentially valuable research.

The report also complains that the law would punish doctors for trying innovative treatments for certain diseases and force them to practice "defensive medicine". Perhaps worst of all, the law would not be as well prepared to adapt itself to new research developments as would a panel of scientists. However, the MPG paper recognizes as "irresponsible" procedures such as the cloning of human beings

or interspecies hybridization.

The DFG, in a more general statement, called for limited and strictly regulated exceptions to the proposed law. DFG President Hubert Markl said that one cannot discount the possibility that such research might continue anyway despite the harsh penalties. Markl criticized the irresponsibility of forbidding research in West Germany that will probably be done elsewhere. He said it would be "almost immoral" to let another country do the research and then use the results.

The MPG and the DFG both point out that the proposed law is inconsistent with West Germany's liberal abortion law. "It seems inconsistent to researchers," said Markl, that 100,000 abortions are performed each year but that the law "forbids the use of an embryo in a research experiment that will help other people".

Steven Dickman

Money from supermarket chain ploughed back into plant research

London

IN what is almost certainly the largest private gift ever made in Britain to support the plant sciences, Mr David Sainsbury, of the grocery family, is to give £15 million, through the Gatsby Charitable Foundation which he endowed, to support research in molecular plant pathology. The money will be used to set up, and support for at least ten years, a new international laboratory located at the John Innes Institute in Norwich. The first staff will be appointed later in the academic year, and work should start in October.

Announcing the gift, Mr Sainsbury, finance director of J. Sainsbury PLC, said that molecular plant pathology was currently an area of great scientific opportunity and offered a significant hope of producing practical benefits, in reducing the cost of crop plant disease, that would be of importance for both industrialized and developing countries.

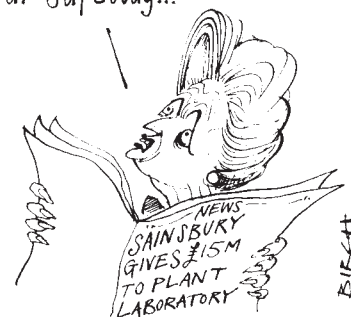
The object of the initiative, developed from collaboration between the Gatsby Foundation, the John Innes Foundation, the Agricultural and Food Research Council (AFRC) and the University of East Anglia, is to provide long-term support for scientists at the highest international level of achievement. The new laboratory, which plans to pay the salaries needed to attract scientists of outstanding ability, is intended as an expression of confidence in the quality and value of British science at a time when many young scientists are tempted to work abroad.

The John Innes Institute, a partner member of the AFRC's Institute of Plant Science Research, was chosen as the site of the new laboratory because of its existing strengths in the study of bacterial and

viral diseases of plants by the techniques of molecular biology.

The Sainsbury laboratory will be based on small core of semi-permanent staff, intended to be "highly innovative scientists", whose studies of host-parasite interaction will be funded in the long term. But there will also be provision for a large proportion of research students,

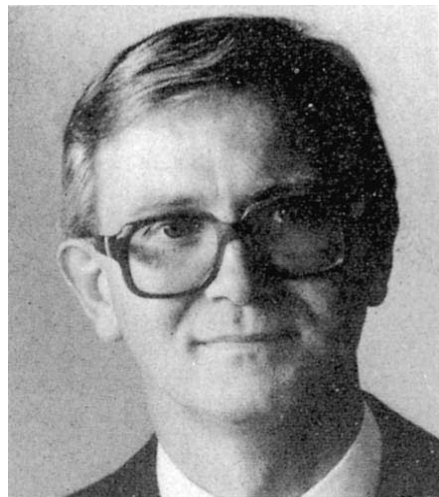
I wouldn't know - I shop at Sainsbury...



visitors and research fellows among the projected thirty or so staff. Some staff seconded to the laboratory will have their salary paid to enable their home institution to replace them. Individuals will decide whether to pursue the practical applications of their research, which will be made widely available in a way that returns benefit to the laboratory itself.

Administration will be guided by a council, on which the three leading scientific staff will serve with a panel of independent scientists of international stature, chaired initially by Professor Harold Woolhouse, Director of the John Innes Institute. The overall aim will be to give active scientists a decisive role in management.

Giles Courtice



"Self-regulation is more effective than passing a law", says DFG president Hubert Markl.

human embryo or out of human and animal germ-line cells are similarly punishable. Experiments on aborted fetuses or eggs fertilized *in vitro* without the intention to implant the egg in a woman's uterus are punishable by up to three years in prison.

The proposed law goes much further than recommended by a series of parliamentary committees. "Not everything that is technically possible should be allowed," Engelhard has said. The Justice Ministry also intends to pass a law against "mothers-for-hire".

The two science organizations want to keep jurisdiction over embryo research under the control of doctors and scientists.

Australian tax strategy the hope for research boost

Sydney

PROponents of Australia's innovative scheme to stimulate industrial research and development through massive tax concessions are enthusiastic about its performance. The latest estimates from the Department of Industry, Technology and Commerce (DITAC) suggest that total private enterprise research and development expenditure has risen by 43 per cent in real terms in the first year of the scheme's operation.

The tax concession, introduced in July 1985, allows a company to deduct A\$1.50 from its taxable income for every dollar spent on research or technical development. As the maximum rate of corporation tax is 49 per cent that means, for a company in profit, that for each dollar spent on research and development a company need spend only 26.5 cents. Research and development is generously defined and includes support activities, such as industrial design, operations research and software development.

Kevin Bryant, director of indicators and resource analysis within the Department of Science, claims the increase in research

and development expenditure is indicative of revolutionary changes in attitude. Research and development expenditure actually began to rise before the tax scheme was in place, with a 19 per cent increase scored in 1984-85. But Bryant believes that many companies began to gear up to take advantage of the scheme as soon as it was announced in December 1984. And since then the pace has increased.

The tax concession has also changed corporate culture, according to John McKenna, director of research and development policy within DITAC, by presenting accountants with a strong reason to take note of research.

Problems do remain, however, and the scheme is still thought to be underexploited. Michael Johnson, a Sydney-based taxation consultant, estimates that there are 80,000 companies expected to be carrying out some research and development. But only 2,100 have registered their intention to take advantage of the scheme. One problem, Johnson says, is that the tax benefit is a corporate one and of no direct benefit to the research departments themselves.

Charles Morgan

Japanese mill given credit for stealthy Soviet subs

Washington and Tokyo

JAPAN'S Prime Minister, Yasuhiro Nakasone, on an visit to Washington last week aimed at reducing Japanese-US trade friction, found that it is not just exports to the US that are worrying officials. At a National Press Club luncheon he was brought to task over exports to the Soviet Union of machinery that is helping Soviet submarines evade US coastal defences.

At issue is the export of four huge milling machines, and the computer program to control them, by Toshiba Machine Company. The machines can mill complex shapes in steel and it is claimed that the Soviets have used them to produce sophisticated submarine propellers that generate very little noise. From last year, US sonar systems began to detect Soviet submarines that were very much quieter than those found earlier, and extremely difficult to track.

Nakasone called the incident "very regrettable" and said that the police would investigate the matter as a criminal offence. Both the milling machine and its program are covered by COCOM regulations designed to prevent high-technology sales to Eastern bloc countries. Police in Tokyo have already searched the head offices of Toshiba Machine and the Ministry

of International Trade and Industry claims that false information had been given by the company on export documents. But officials accompanying Nakasone say that key information for silencing Soviet submarines came not from Japan, but from a US spy ring.

Alun Anderson & David Swinbanks

Soviets out of ODP

Washington

To the dismay of participants in the multinational Ocean Drilling Program (ODP) meeting in College Station, Texas, President Ronald Reagan has decided that the Soviet Union should not be invited to become a member of ODP. The Soviet Union had been asked to join two years ago, and a US delegation was poised to go to Moscow last February for a formal signing ceremony. But at the last moment, the Pentagon raised national security concerns because Soviet scientists would have access to sophisticated technology aboard the drilling ship. Officials at the National Science Foundation claimed there was no basis for such concerns, but were overruled by the president and his National Security Council.

Joseph Palca

Another try for research push

London

A NOVEL centre to coordinate the commercial exploitation of current scientific research while encouraging new directions is in its final stages of planning. Its brief is to pick 'winners' for British industry. The institute, the brainchild of the Cabinet Office and the scientific advisors to the government's ACARD (the Advisory Council for Applied Research and Development), is expected to be housed at one of Britain's top science universities. Imperial College in London, Cranfield Institute of Technology in Bedfordshire, Warwick University in the Midlands and the London-based Technical Change Centre are among the front runners.

Sir Francis Tombs, chairman of Rolls Royce and ACARD, has managed to acquire much of the money needed to create the centre, a budget in the region of £5 million. The Tombs approach was highlighted in an ACARD report of last year which concluded that: "A process is needed to prioritize and guide a substantial proportion of that part of the national scientific resource be it Research Councils, Ministry of Defence or Department of Trade and Industry, and to stimulate its effective exploitation to the benefit of the United Kingdom".

Economic and management consultants Segal Quince Wicksteed, who have expertise in the commercialization of research, were appointed last year to steer ACARD, and the government, towards the creation of the centre. The consultancy has completed its study, from which an outline of the new centre has been derived.

In some respects the new centre will perform some of the tasks of the American Office of Technology Assessment which advises Congress but the British model will be independent from government. The Japanese MITI (Ministry of International Trade and Industry) which develops much of the successful policies behind Japan's industrial expansion has also inspired the British.

There have been several previous attempts to bring industry to the attention of university researchers and vice versa. The National Enterprise Board (NEB), which held substantial stakes in the British industries that needed investment, and the National Research Development Corporation (NRDC), which encouraged start-ups, were merged into the British Technology Group (BTG), supposedly to help exploit the scientific research effort. The current government has never disguised its dislike of the BTG and curbed its influence by encouraging academics to exploit their own research. **Bill Johnstone**

AIDS tests upset haemophiliacs

Tokyo

If you were going to have a tooth extracted or give birth would you expect to be tested for AIDS — acquired immune deficiency syndrome? It may seem extreme, but in Japan if a doctor suspects merely on the basis of an interview that you may be infected with AIDS virus that is precisely what he should do according to a guidebook issued by the Health and Welfare Ministry to hospitals and medical practitioners throughout Japan. The National Association of Haemophiliacs last week demanded withdrawal and revision of the guidebook, on the grounds that it may lead to a violation of human rights. At issue is a section which deals with prevention of AIDS transmission in hospitals.

According to the guide, treatment of any patient should be divided into three levels according to the amount of blood likely to be shed, namely, no blood shed (for example, injections, blood sampling, drip), slight blood shed (biopsies, tooth extractions, fibre optic probe analysis), and heavy blood shed (operations, birth, dialysis). If the doctor suspects on the basis of an interview that a patient to undergo treatment in the latter two categories may be infected with AIDS, he can carry out an antibody test, if he thinks it is needed. But no mention is made of informing the patient or obtaining consent.

Yukio Yasuda, vice-president of the Tokyo branch of the haemophiliac association, points out that the lack of a clause for informed consent runs completely counter to the World Health Organization's recommendation issued in April last year which states that even in the case of diagnosis of a suspected AIDS patient tests should not be carried out without the patient's consent. And he fears that the guide may lead to refusal of treatment for carriers and violation of the privacy of ordinary patients. The guidebook is of particular concern to haemophiliacs as a large percentage of Japan's AIDS virus carriers are haemophiliacs who were infected through imported blood products — in a recent survey of 187 carriers, 85 per cent were suspected to have been infected through imported blood.

An official of the Health and Welfare Ministry dismissed the suggestion that the guide would lead doctors to carry out tests without the patient's consent, because, he said, doctors usually explain treatment to patients. But it is well known in Japan that tests for viruses such as hepatitis B, adult T-cell leukaemia and syphilis are often carried out without consent.

David Swinbanks

Indian dam enthusiasm is dampened by Soviet burst

New Delhi

THE recent dam-burst in the Soviet republic of Tadjikistan has dampened Indian enthusiasm for the Soviet Union's offer to build a dam on the Bhagirathi, the Himalayan river, in a seismic zone of northern Uttar Pradesh. If built, at a cost of \$1,000 million, the dam would be 266 m high and generate 2,000 MW of electricity, but

Let them
have dams...



would flood several thousand hectares of Himalayan forest and displace 70,000 people centred on the township of Tehri.

Despite opposition from environmentalists who argue that the filling of such a large dam, sited over tear-faults, might trigger earthquakes and put 250,000 lives at risk, the government has decided to go ahead now that \$200 million has already been spent on the project.

A Soviet team recently visiting Tehri

declared the project feasible and offered to carry it through on a turnkey basis. But a Soviet invitation to visit the Nurek and Begun dams in the Voksh valley, built with the planned technology over geological faults, provides little reassurance in the wake of the dam-burst in that area. A local citizens' group has now taken the issue to the Indian supreme court.

Tehri dam is not the only hydroelectric project meeting opposition on environmental grounds. A \$1,200-million World Bank project on the Narmada is in trouble, and a \$500 million project on the Indravati has been rejected on the grounds that it would destroy tribal forests. Hydroelectric dams have always been contentious in India. Even so, the government has been entering into bilateral agreements in the hydroelectric sector in the hope of bridging the 20,000-MW power gap expected by the end of the century.

A French company has won the turnkey contract to build the \$700-million Dulhasti project in central India. A Japanese-Italian consortium is bidding for the Thein dam in the Punjab, a Swedish company (Svenska Umentgiuter) for the 480-MW dam at Uri in Kashmir while Canada has offered to build a variety of hydroelectric and thermal electricity projects. But Indian environmentalists are beginning to ask why donors help to uproot people and trees.

K. K. Jayaraman

The Byelorussians had a word for it

London

A REQUEST from 28 eminent Byelorussian writers and scholars for their language to have a proper scientific vocabulary, free of "unjustified foreign borrowings" has received an unsympathetic answer from the Party authorities. The issue of scientific vocabulary was raised in an "Open Letter to Gorbachev", which has been circulating privately since December, and is part of a package of measures proposed for "the radical improvement of the position of the national language, culture and patriotic education in the USSR". The signatories are not dissidents — they include members of the Byelorussian Academy of Sciences.

According to the open letter, the status of the Byelorussian language has been consistently declining under an official policy of switching to Russian as the language of the education system. The absence of a native scientific vocabulary is a symptom of this process. It is not that Byelorussian has never had words for new scientific concepts or has borrowed them from the international Graeco-Latin stock. When the signatories of the letter speak of

"foreign borrowings", they mean Russian forms. To take one example, a 1923 glossary of Byelorussian technical and scientific terms includes *prostakutnik* — "rectangle". The 1982 Russian-Byelorussian dictionary, however, replaces this with *pramavuholnik*, the Russian word rendered in Byelorussian orthography.

The official answer to the petition came from the First Secretary of the Communist Party of Byelorussia, J.J. Sokalau, in a report to the Central Committee Plenum. Although he did not refer directly to the open letter, he castigated the "creative and scientific workers" who have "concluded hastily" that an "incorrect attitude" has been adopted to national languages. "Leninist principles" he said, have brought about a situation where virtually the whole population of the BSSR understands both Russian and Byelorussian, and where no one is prevented from conversing with a friend or speaking from a rostrum in Byelorussian if he or she so chooses. Those who "make mistakes" in understanding these matters, Sokalau said, "must be helped to sort things out". Vera Rich

Coastal waters in jeopardy

Washington

WITHOUT some intervention, coastal waters and estuaries around the United States will become increasingly polluted. But efforts to evaluate current conditions and predict those in the future are hampered by a paucity of data from many regions of the country, says a report released last week by the Congressional Office of Technology Assessment (OTA).

Curtailling waste disposal activities and runoff is the only clear choice for improving the quality of estuaries and coastal waters, according to the report. One simple solution is to move dumping activities further off-shore, but this presents technical, logistical and economic problems. Reducing waste generation, recycling wastes and treating waste material all alleviate damage to the environment, but cannot solve the problem entirely.

The slightly better news is that the open ocean is in better shape than the coastal environment. In contrast to estuaries and coastal waters, relatively little dumping and discharge takes place at sea, although the report predicts there will be increasing pressure to dump sewage sludge off-shore, rather than in coastal waters. Evaluation of the impact of open ocean dumping is also hampered by lack of information. Nicholas Sundt, an analyst for OTA, says that poor information could be masking problems.

Although water quality in certain coastal waters and estuaries has improved in the past, these have typically been the ones in the worst shape, says Sundt. Economic conditions have forced many areas to abandon treatment. Expensive though treatment programmes were in the past, new efforts will cost even more. Since communities are often far removed from the effects of their disposal activities, it can be hard to generate political support for solving the problem.

The situation is not hopeless. Congress this year passed an act authorizing \$18,000 million in grants and loans for sewage treatment facilities. The Environmental Protection Agency (EPA) is developing management programmes for coastal waters and estuaries. But there is no integrated national strategy for improving water quality. With its penchant for understatement, the OTA report states that given the threats to these waters, "it now seems appropriate that Congress and EPA begin developing a systematic framework to implement the water quality approach more extensively." Joseph Palca

The report "Wastes in Marine Environment" is available as OTA-O-334 from the US Government Printing Office.

Oil explorers make waves in Alaskan tundra reserve

Washington

US Interior Secretary Donald Hodel has enraged environmentalists in two controversial moves designed to open up new areas to oil exploration. Last week he recommended to Congress that oil development be allowed in the coastal plain of the Arctic National Wildlife Refuge in Alaska. A few days later, he followed that with a five-year plan that would allow offshore drilling in areas off the California, Florida and Alaska coasts.

In recommending drilling in the Arctic National Wildlife Refuge he went beyond an earlier Interior Department report (*Nature* 324, 401; 1980) that argued for delaying development in a part of the refuge used for calving by the 18,000-head 'Porcupine' caribou herd. Hodel now claims the report was in error in saying the herd would be harmed by oil development.

Susan Alexander of the Wilderness Society, one of several conservation groups opposing development, said that it was a case of "altering facts that didn't support development goals". The area, she says, is one of the most extensive

undeveloped landscapes left in North America. Now, conservationists will aim to "educate the Congress and the American public so they'll feel the same way about the refuge as they do about the Grand Canyon or Yellowstone".

Business and oil industries praised the shift in Alaskan policy but were much less happy about the new plan for offshore drilling. Despite opening what environmentalists regard as some of the "most sensitive coastal and marine areas" to oil drilling it stopped far short of an earlier Interior Department proposal that the entire continental shelf be made available.

Both the Alaskan and offshore proposals will now face fierce battles in Congress. The oil industry will be arguing that new finds are essential to halt the increasing dependence of the US on foreign oil supplies. But a final decision on the Alaskan refuge is unlikely until after the next Presidential election given the number of voters who favour conservation. And if Congress does not reject the offshore drilling plan it can block drilling leases later. **Alun Anderson**



Developer pays for King's College move

London

A NOVEL kind of collaboration between UK universities and commerce has emerged. Mr Kenneth Baker, Secretary of State for Education and Science, has agreed that King's College London (KQC) should enter into a partnership with a property developer to secure a new site for itself. In return for financing the purchase of the lease on an empty government building, the commercial partner will be allowed half the profits from the development of the college's existing properties.

KQC has been formed within the past four years by the amalgamation of the old King's College with two smaller units of the University of London, Queen Elizabeth College and Chelsea College, whence KQC. Academic development has been impeded by the lack of space on the main site in the Strand. But earlier this year, Baker said the government would not provide special assistance to the college for

purchase of a lease on Cornwall House, across the Thames from the Strand site.

Under the deal now struck, the commercial developer will put up the £1.25 million required to purchase the lease of the building. The college will then have four years in which to raise the funds needed to fit the building for its new purpose. The hope is that the funds required, which may amount to £20 million, will come from using the vacant sites for other purposes. One of the sites concerned occupies 4.5 acres of Kensington in west London, an expensive residential district, where a piece of road big enough to park a single automobile is worth £10,000.

This development suggests that the government appears prepared to contemplate radical changes of the rule that the proceeds of the sale of academic properties should be used exclusively for academic purposes (or even returned to the Treasury). □

Still shrouded in mystery

SIR—Like most observers keen to know the historical provenance of the Shroud of Turin, I welcome the decision to subject the relic to radiocarbon dating. However, clouds loom on the horizon, in the form of confusions about the protocols for the tests. The procedures as so far understood involve a number of samples of the shroud which are to be divided among as many as seven laboratories. These laboratories will be asked to date dummy samples along with the shroud, and none will even know which of their samples are from the Turin relic. This blind procedure will avoid any possible taint of prejudice on the part of the testing laboratories.

However, such a protocol leaves serious unanswered questions about the possibility of tampering with the samples themselves. How are independent observers to know whether any of the samples which testing laboratories receive are in fact actual linen fragments from the shroud? Are we simply to take the Vatican's word for it? Repeated enquiries in this matter made by me and by the US Committee for the Scientific Investigation of Claims of the Paranormal have so far elicited no satisfactory answers. One prominent shroud authority, Father Peter Rinaldi, has given assurances that the British Museum is acting as "guarantor" of the tests. But the relevant person in the British Museum, who was in fact present at the meeting in Turin last autumn which recommended the testing procedure, has declined to divulge any information about testing protocols because of "confidentiality". He has referred correspondents to Cardinal Ballestrero in the Vatican and to the Pontifical Academy of Sciences. Inquiries there have so far gone unanswered.

The situation as it now stands is most disturbing. After years of discussion, there is agreement to go forward with ^{14}C tests on the Shroud of Turin, but apparently so far without due regard for an open disclosure of procedures for taking the samples. Evidence for or against the authenticity of a relic of such widespread veneration involves deep religious passions: for some people there is a great deal potentially to be lost. So there must be no hint that, for example, fibres of mummy linen might have been supplied to the laboratories, rather than actual shroud samples. If those conducting the tests wish the results to be taken seriously, they must offer their procedures to open inspection by independent observers. "Confidentiality" is out of the question.

DENIS DUTTON

*School of Fine Arts,
University of Canterbury,
Christchurch 1, New Zealand*

CORRESPONDENCE

UK research funding

SIR—Selectivity of research funding is clearly here to stay but that does not mean that we have to accept the current mechanisms for the distribution of research funds. Is the University Grants Committee (UGC) the correct body to implement the policy? There are three reasons for believing that it is not. The first is the widely held perception that its criteria for selection are invalid. In a recent analysis of UGC rankings in my own discipline, psychology, for example, it was found that the only good predictor of ranking was department size (R. Gillet, *Bull. Br. Psychol. Soc.* **40**, 42; 1987). The second is the concentration of decision-making in a few hands, which means that judgements are inevitably taken by people of limited expertise. The third is that because the UGC deals with institutions rather than individuals, it is excessively inflexible. If the present exercise continues, it will mean that, in a few years, people with good ideas in 'second-class' institutions will have little chance of putting them into effect while those with mediocre ideas in 'first-class' institutions will find them financed.

The only way round these problems is to abandon the now outdated notion of dual funding for research and change to the US system of full funding of research expenses and overheads via project grants.

The presumption would be that all state-financed university and polytechnic employees are hired to teach. They would gain time for research by using research funds to buy out parts of their salaries. This would give great flexibility, provide individual scientists with some control over their own careers and place decision-making in the hands of the expert panels of the research councils, charities and other funding bodies. It would also mean that institutions seeking industrial sponsorship in preference to research council funds would not be penalized.

CHARLES R. LEGG

*City University,
Northampton Square,
London EC1V 0HB, UK*

Chinese dissent

SIR—Expelled in January from his position as vice-president of the University of Science and Technology of China (USTC, see *Nature* **325**, 290; 1987), Professor Fang Li-Zhi, criticized as a "Chinese Sakharov" by a senior official of the Communist Party of China (CPC), now has to leave USTC in Hefei to work in Beijing. And the president of USTC, Professor Guan Wei-yan, was replaced by the ex-deputy-minister of propaganda of the Central Committee of CPC, Professor Teng Teng. This may be a result of the "anti-bourgeoisie democratization struggle" espoused by the CPC leadership.

Mr Deng-Xiao-Ping said on 13 January 1987, when he received a Japanese delegation, that Fang is a "bad man" who has "polluted the young students with the ideology of capitalism," resulting in student demonstrations in more than 170 Chinese universities in support of more democracy and the liberty of the press.

Fang, who is 50 years old, is an eminent theoretical astronomer and member of the consultation committee of the International Centre for Theoretical Physics and the International Centre for Relative Astronomy. He won the ICRA prize in 1985 for his work in this field.

Fang thought it his duty as a scientist to express his opinion when faced with injustice. As a former student of USTC, I consider Deng's decision to remove Fang was a step backwards in the move towards reform in China that he himself initiated. It will be regretted by Chinese intellectuals and the Party.

ZU-FENG XU

*LTE/UTC BP 233,
60206 Compiègne, France*

Theoretical threat

SIR—Those of us who have been engaged in the struggle against creationism in Queensland can take some small measure of comfort from the letter from the Minister for Education, Mr Lin Powell (*Nature* **324**, 204; 1986). We have been trying to get a clear statement about what is meant by "balance". It now appears that it is sufficient for teachers to acknowledge that there are "beliefs deeply held by a significant proportion of the community", which are not scientific in nature, and hence should have no part in any science class. This is likely to be unpalatable to the local creationists, as it would only take a minute or two of the 10 hours on evolution.

But we still have confusion and misunderstanding about the nature of a scientific theory. In browsing through an old volume of *Nature*, I came across an article, by Julian Huxley (**163**, 941; 1949) that said: "Throughout the discussion, Lysenko and his followers treat neo-Mendelism (or Morgeno-Mendelism or whatever other title they apply to modern genetics) as a mere theory, in the sense of a hypothesis, not in the usual sense in which it is used in science, of a set of conceptions tying together a vast body of experimental results and established laws..."

Creationists (and Powell) seem to use the word "theory" as it was used in Stalinist Russia. Let us hope there is a change of mind, before agriculture in Queensland suffers in the way that agriculture in the Soviet Union did in the 1950s.

KEN SMITH

*Department of Mathematics,
University of Queensland,
St Lucia, Queensland 4067, Australia*

Glassy solids come of age

There is a promise of some progress in the understanding of the differences between glasses on the one hand and other kinds of solids, or liquids, on the other.

Is the glassy solid about to come of age? This is the possibility suggested by the apparently boundless interest, just now, in disordered conditions of the solid state, the manifestations of which apparently multiply as the weeks go by. Thus the possibility that quasicrystals, the structures of solids with apparently five-fold symmetry, are glassy structures remains (see M. Widom's News and Views article on page 19 of this issue).

So does the possibility that the new ceramic superconductors, with their non-stoichiometric chemical composition, may be solid solutions of one phase in another, with clumps of one phase extending over very small numbers of inter-atomic distances. There has also been some gentle excitement about the amorphous forms of ice formed at high pressure (see last week's *Nature* **326**, 823; 1987). In the circumstances, it is more than ever a pity that so little has been done to put the theory of glasses on a footing that is at once firm and accessible.

There is no obvious room for complaint. Nobody will claim that the problem of understanding what a glass is like can be simple. Indeed, the definition we were all given at school, that a glass is a liquid capable of supporting a shear stress, is still about the best there is; the obvious defect is that this phenomenological statement by itself provides no pointers to the atomic structure of the glassy state.

The other conspicuous part of our schoolroom lore, that glasses are inherently unstable relative to some more ordered solid state, and remain metastable only for kinetic reasons — the transition to crystallinity is exceedingly slow — cannot apply to all glasses, polymer glasses, for example, where separate molecules may be inextricably tangled with each other, so that the solids are irretrievably disordered.

But there is one practical sense in which the formation of glasses is a kinetic phenomenon: to turn a supercooled liquid into a glass, the rate of cooling has to be great enough to ensure that some degree of departure from equilibrium will be incorporated into the solid.

None of this implies that people have been indifferent to the need for understanding. That there is something special about the glassy state, and therefore much in need of definition and description, has been clear for many years, not merely because people have been asking how it

can be that materials that can support shear stress nevertheless creep, if comparatively readily (which is why windowpanes in old houses are thicker at the bottom than the top), but because of empirical observations at low temperatures which, among other things, show that the specific heat is a linear rather than a Debye-type cubic function. (The moral is that there is more scope for sucking up low-energy quanta in a glass than in a classically vibrating lattice.)

The wind now seems to be blowing in a direction much favoured for much more than ten years — towards that picture of the microscopic structure of a glass in which small well-ordered groups of atoms are oriented relative to each other in a random fashion and, therefore, connected together by equally random bonds. This arrangement has the virtue of being in agreement with X-ray diffraction measurements to the extent that there is short-range order of the kind found in powder diffraction photographs, but no more long-range order than can be found in a liquid. (Resistance to shear-stress arises from the mere existence of linking bonds.) The obvious snags are that randomly arranged bonds may imply severe local energetic penalties and that there are geometrical problems as well; often the natural small-scale units will be tetrahedral units, but there is no way of filling space with structures like that, any more than there is a way of tiling the two-dimensional plane with pentagons.

The much more serious difficulty is that there is no obvious way of calculating the properties of a glass built thus from small ordered groups of atoms. What conviction can a theory carry if it cannot be used to make some kind of prediction, even of the properties of some well-studied glass? That, for ten years, has been the hang-up. Now, with a little luck, there may be a way out of the fix.

R. Kree of the University of Düsseldorf, L.A. Turski of the Polish Academy of Sciences and A. Zippelius of the solid-state institute at the West German nuclear research centre at Jülich in Nordrhein-westfalia have just published (*Phys. Rev. Lett.* **58**, 1656; 1987) a seductive account of how such a problem might be handled. If a glass is a system in which well-ordered groups of atoms which are randomly oriented interact with identical neighbouring groups of atoms by means of randomly drawn bonds, why not rep-

resent the entire system by that elaboration of the Ising lattice (a three-dimensional lattice with either parallel or anti-parallel magnets on every vertex) called the Potts model, in which the interacting elements at the vertices may have a denumerably infinite number of orientations?

To be sure, the simple problem of the general Ising lattice has not yet been solved, but there are tricks (computational, but also topological) that can finesse that difficulty.

Kree and his associates give an impressive prospectus of their achievement. What Ising lattice people attempt is usually the calculation of the thermodynamic partition function, from which the usual thermodynamic quantities can be derived, from a statement of the geometry of the problem. Kree *et al.* build into their analysis a consideration of the interaction between the local orientation of a group of atoms in a glass (from which it may be possible to calculate the shear strength of glasses and its variation with temperature), which has always been a technical option from which most of the practitioners have shrunk.

Tantalizingly, the details of the calculations are not yet available, but only the general conclusions claimed for them. Briefly, the narrative continues, there is such a thing as the transition to a glassy state, the transition temperature is a function of the degree of disorder frozen into the solid but there is no latent heat at the glass transition, whether or not there are other discontinuities, nor is there a change of specific volume.

All of us will live more happily with these conclusions when the intricacies of the argument have been written up. No doubt, that will not be long from now. Meanwhile, those working in the field will be reflecting that, while the Potts model as such is so hard to handle that it offers very little in the way of rapid progress, the Ising lattice has yet again been shown to be a durable and adaptable model of a huge variety of physical systems. Now the obvious need is to find a physical system, a real glass, that will conform with what Kree *et al.* promise. The plain truth is that Kree *et al.* have suggested powerfully a way in which a familiar model of the crystalline state may be adapted to an understanding of puzzling disorder. Watch this space, as they say.

John Maddox

Immunology

Cytolytic T-cell melodrama

Pierre Golstein

How can some T cells lyse other cells *in vitro*? The currently most popular view is that of a murder, the cytolytic T cells somehow sending pore-forming molecules to target cells which die as a direct consequence. The challenging opinion sees the cytolytic T cells as inducing the target cells to commit suicide. This opinion receives strong support from a report by David S. Ucker on page 62 of this issue¹ showing that a mutable element within the target cells governs their lysability by T cells or by glucocorticoids. Which precise mechanism is at work may well be of great practical importance, as lysis by T cells *in vitro* probably mimics the disposal by T cells *in vivo* of virus-infected cells and possibly of tumour cells.

The current quasi-dogma, based on pore-forming molecules (*a* in the figure), stems from striking apparent analogies between lysis by T cells and lysis by soluble factors of the complement system. First, fractionation experiments^{2,3} led to the discovery in cytolytic T and natural killer (NK) cells of pore-forming, lytic molecules called perforin or cytolsin, with similarity⁴⁻⁶ to the lytic pore-forming C9 component of the complement system. Second, the presence of several serine esterases, identified either at the protein⁷ or at the nucleic-acid level, mostly (but not only) in cytotoxic T and NK cells, is very reminiscent of the presence of other serine esterases at several steps of the complement maturation cascade. These results made a pore-forming, complement-like mechanism of T-cell killing a reasonable bet.

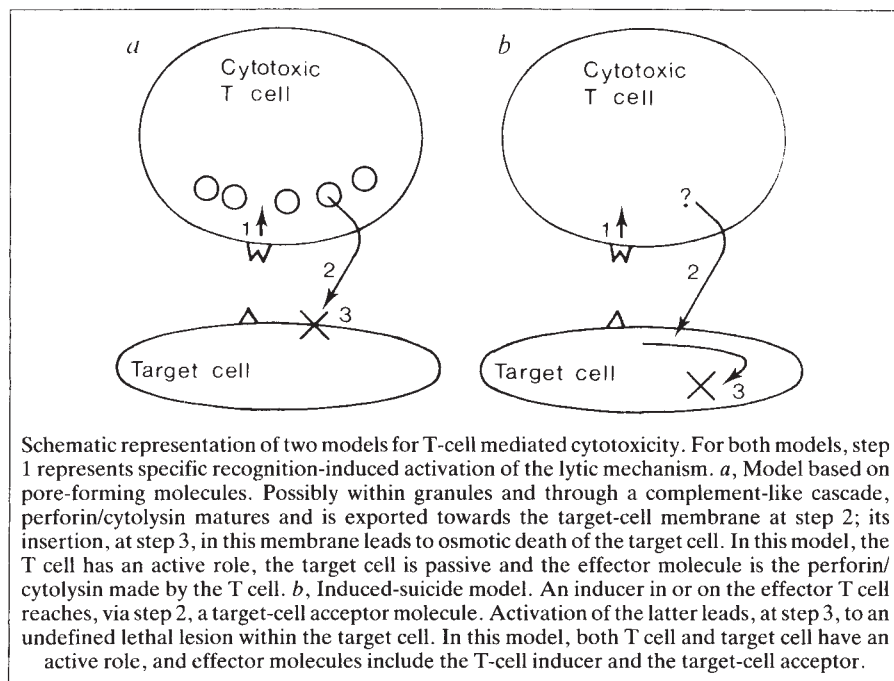
A few disquieting observations or questions have emerged, however. First, antibodies that inhibit perforin-mediated lysis do not inhibit T-cell mediated (as opposed to NK-cell mediated) lysis. Second, what protects the cytolytic T cell itself from being lysed by the pore-forming protein it produces? Third, no perforin activity was detected⁸ in highly cytotoxic, sensitized T cells from the peritoneal exudate. Fourth, and relevant to what follows, when target cells are subjected to cytolytic T cells, it is their nuclear, not cytoplasmic, membrane that is altered first⁹, with rapid DNA fragmentation¹⁰. Interestingly, corticosteroids induce similar phenomena in thymocytes¹¹. How can the first detectable effect of an external toxic agent be the lysis of an internal membrane?

The alternative, then, was to postulate⁹⁻¹¹ that an internal auto-destructive mechanism is triggered (*b* in the figure). In line with this concept, Ucker in this issue¹ now

shows that, although a given thymoma cell clone is sensitive to both corticosteroids and T-cell mediated cytotoxicity, a variant clone derived therefrom is resistant to both (but not, significantly, to complement-mediated lysis). He checked resistance by cloning efficiency, ⁵¹Cr release and DNA fragmentation assays. The striking difference between parent and variant is not accounted for by any detectable difference in recognition pathways (that is in the amounts of glucocorticoid

ability of the cell to die, perhaps relevant to the programmed death postulated in the thymus and in some forms of cell senescence.

The increased possibility of an induced suicide mechanism may call for a modification of fractionation strategies (see figure legend). It may be necessary to look, not or not only for a cytolytic molecule in the effector cell, but for inducer molecule(s) in the effector cell and an 'acceptor' molecule (as inferred from Ucker's work) in target cells. Another implication comes from previous observations that cytolytic T cells can themselves serve as targets; their postulated inducer and acceptor molecules might have to reside in different cellular compartments if these cells are to live at



Schematic representation of two models for T-cell mediated cytotoxicity. For both models, step 1 represents specific recognition-induced activation of the lytic mechanism. *a*, Model based on pore-forming molecules. Possibly within granules and through a complement-like cascade, perforin/cytolsin matures and is exported towards the target-cell membrane at step 2; its insertion, at step 3, in this membrane leads to osmotic death of the target cell. In this model, the T cell has an active role, the target cell is passive and the effector molecule is the perforin/cytolsin made by the T cell. *b*, Induced-suicide model. An inducer in or on the effector T cell reaches, via step 2, a target-cell acceptor molecule. Activation of the latter leads, at step 3, to an undefined lethal lesion within the target cell. In this model, both T cell and target cell have an active role, and effector molecules include the T-cell inducer and the target-cell acceptor.

receptors on one hand or antigen-specific binding on the other — and the fact that lectin bypass of T-cell recognition still leads to no lysis of the variant cell). Moreover, a rare spontaneous revertant cell could be isolated, through its sensitivity to glucocorticoids, which is also sensitive to T-cell mediated cytotoxicity. Thus, a parent cell is sensitive to both corticosteroids and T-cell mediated cytotoxicity, a variant cell is resistant to both, and a rare revertant is sensitive to both.

A minimal conclusion from these results is that a mutable target-cell molecule, at a stage apparently distinct from recognition by corticosteroids or T cells, controls target-cell lysability by either. This molecule would not be required for cell life (because a variant cell where it is mutated can still multiply) but would be required for certain varieties of cell death (as the same variant cell cannot be lysed any more by certain otherwise lethal agents). This putative molecule may have a more general function related to the

all. Finally, which role would then remain for the T/NK cell pore-forming proteins — a decoy masking the induced suicide mechanism, or perhaps an alternative killing system operating as a function of the particular effector and/or target cells at play? □

1. Ucker, D. S. *Nature* **327**, 62–64 (1987).
2. Henkart, P. A., Millard, P. J., Reynolds, C. W. & Henkart, M. P. *J. exp. Med.* **160**, 75–93 (1984).
3. Podack, E. R. & Konigsberg, P. J. *J. exp. Med.* **160**, 695–710 (1984).
4. Zalman, L. S., Brothers, M. A., Chiu, F. J. & Müller-Eberhard, H. J. *Proc. natn. Acad. Sci. U.S.A.* **83**, 5262–5266 (1986).
5. Tschopp, J., Masson, D. & Stanley, K. K. *Nature* **322**, 831–834 (1986).
6. Young, J. D., Liu, C., Leong, L. G. & Cohn, Z. A. *J. exp. Med.* **164**, 2077–2082 (1986).
7. Pasternack, M. S. & Eisen, H. N. *Nature* **314**, 743–745 (1985).
8. Berke, G. & Rosen, D. *Transpl. Proc.* (in the press).
9. Russell, J. H. & Dobos, C. B. *J. Immun.* **125**, 1256–1261 (1980).
10. Duke, R. C., Chervenak, R. & Cohen, J. J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6361–6365 (1983).
11. Cohen, J. J. & Duke, R. C. *J. Immun.* **132**, 38–42 (1984).

Pierre Golstein is at the Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, CNRS, Case 906, 13288 Marseille Cedex 9, France.

Pterosaur locomotion

Joggers or waddlers?

David M. Unwin

PTEROSAURS were the first vertebrates to achieve true active flight, and they dominated the skies for more than 140 million years — twice the time that birds have been dominant. Uncrushed remains of pterosaurs are understandably rare as the skeleton of these animals consisted only of thin sheets, bars and hollow tubes of bone. The recent discovery^{1,2} of two pterosaur pelvic girdles, one slightly crushed but complete and the other uncrushed though incomplete, is therefore of special interest. In particular these specimens, together with other evidence, should resolve the long argument about pterosaurs' deportment. The reports demonstrate that pterosaurs did not have a fully erect bird-like stance and gait, with good terrestrial mobility, as some propose. Most, if not all, pterosaurs could manage only a clumsy waddle, and consequently spent most of their lives hanging from trees and cliffs, a lifestyle that resulted from their arboreal origin of flight.

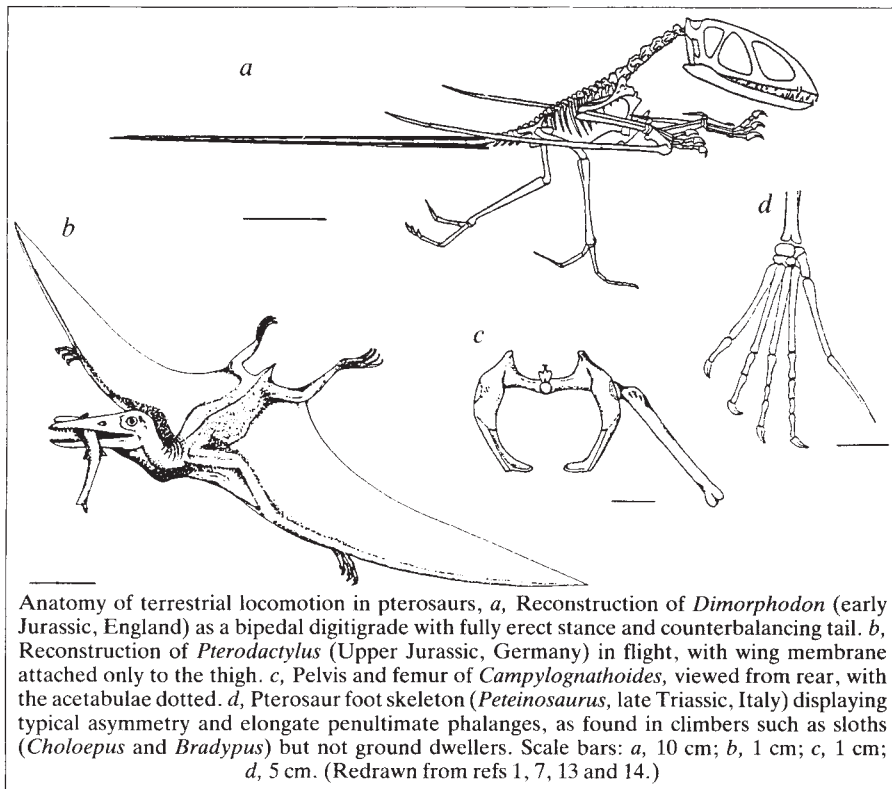
Recently, the traditional concept of pterosaurs as glorified, reptilian gliders has been replaced by a much more energetic concept. Pterosaurs are now generally thought to have been active, warm-blooded animals and highly proficient fliers, represented by small, skimmer-like forms such as *Rhamphorhynchus*, capable of snapping up fish from the water's surface, and giant forms such as *Quetzalcoatlus*, with a 12-m wingspan, magnificently adapted to condor-like, soaring flight. Recent discoveries include the bizarre filter-feeder *Pterodaustro* from the late Jurassic of Argentina; the shell-fish-crusher *Dsungaripterus* from the Lower Cretaceous of China; and the peculiar, multi-fanged *Cearadactylus* from the late Lower Cretaceous of Brazil. These specimens indicate that pterosaurs were a highly diverse group of animals, playing an important role in Mesozoic food webs and occupying many niches now filled by birds^{3,4}.

Not all aspects of pterosaur life, however, are agreed upon. The most important controversy concerns pterosaurs' mobility when grounded. In the early 1970s it was suggested that pterosaurs had a narrow wing membrane, attached to the body wall, but not (as in traditional reconstructions) to the hind limbs⁵. Freed of this impediment, a reconstruction is possible with the hind limbs beneath the body, giving a fully erect stance like that of birds and bipedal dinosaurs. Advocates of this idea add that pterosaurs stood on their hind limbs alone (the forelimb not being suitable for terrestrial locomotion),

walked on their toes (digitigrade) not the soles of their feet (plantigrade), and thus were capable of fast and efficient movement⁶ when grounded (*a* in the figure). Considerable evidence, drawn largely from uncrushed remains of *Dimorphodon*, a medium-sized, early pterosaur from the Lower Jurassic of Dorset, has been cited⁷

protagonists even draw support for opposing views from the same pelvis of *Campylognathoides* (a small, Lower Jurassic pterosaur)^{8,9}, but as this specimen is badly crushed, the evidence either way remains unconvincing.

Thus, the recent discovery of two relatively uncrushed pterosaur pelvises is of key importance. One specimen is a complete, slightly crushed pelvis of *Campylognathoides* from the Lower Jurassic Posidonienschiefer of Braunschweig near Schandelah, West Germany¹. The other is an incomplete, uncrushed pelvis of a medium-sized pterosaur from the late



Anatomy of terrestrial locomotion in pterosaurs, *a*, Reconstruction of *Dimorphodon* (early Jurassic, England) as a bipedal digitigrade with fully erect stance and counterbalancing tail. *b*, Reconstruction of *Pterodactylus* (Upper Jurassic, Germany) in flight, with wing membrane attached only to the thigh. *c*, Pelvis and femur of *Campylognathoides*, viewed from rear, with the acetabulae dotted. *d*, Pterosaur foot skeleton (*Peteinosaurus*, late Triassic, Italy) displaying typical asymmetry and elongate penultimate phalanges, as found in climbers such as sloths (*Choloepus* and *Bradypus*) but not ground dwellers. Scale bars: *a*, 10 cm; *b*, 1 cm; *c*, 1 cm; *d*, 5 cm. (Redrawn from refs 1, 7, 13 and 14.)

in support of this idea. Most important, the head of the femur is directed sharply inward at about 120° to the shaft, permitting the femur to move in an approximately vertical plane, as it does in birds. *Dimorphodon* also possessed a bird-like knee joint and a mesotarsal ankle joint similar to that of birds and dinosaurs. Its feet were symmetrical about the third toe, as is typical of terrestrial birds, but is not found in any other pterosaur.

Not all palaeontologists accept this idea, some preferring a reconstruction⁸ (*b* in the figure) with the hind limbs splayed out sideways and at least partially attached to the wing membrane, restricting terrestrial movement to an ungainly waddle. A crucial point concerns the orientation of the acetabulum, the socket in the pelvis into which the head of the femur fits. Those in favour of the erect stance maintain that the acetabulum faced outwards and downwards⁶, whereas those who prefer the sprawling stance maintain that it faced outwards and upwards^{3,4}. The

Lower Cretaceous of Australia². In both cases, the acetabulae face outwards and upwards (*c* in the figure). This orientation is also seen in uncrushed, incomplete pelvises of *Rhamphorhynchus* from the Upper Jurassic Solnhofen Limestone of Bavaria¹. So all these specimens support the 'sprawling' hypothesis. The femur could not have swung vertically even if the head of the femur was directed inward at 120°. Furthermore, the heads of most pterosaur femora are obliquely offset at 130°–160° to the shaft⁴ and this, along with the acetabulae facing out and up, implies that the hind limbs of pterosaurs splayed out, giving a clumsy, sprawling gait, encouraging them to seek the safety of the nearest tree or cliff.

Contrary to some views^{6,7,10} in favour of the erect stance, pterosaurs show arboreal and scansorial (climbing) adaptations. As well as a highly elongate fourth finger forming the main wing spar, the forelimb also contained three small fingers, each of which bore a large, hooked and pointed

claw similar to those on climbers such as bats and woodpeckers. The claws were mounted on elongate phalanges that increase their grasping power. It has been argued⁷ that some pterosaurs used their claws for catching prey; but others with equally well developed claws, such as the filter feeder *Ctenochasma*, would have had no such use for them. It could also be argued that claws helped maintain position during mating; but sexual dimorphism in claw size has yet to be demonstrated.

The structure of the foot, too, suggests that pterosaurs were good climbers as the claws, although smaller than on the hand, are also hooked, pointed, and moreover mounted on elongate phalanges (*d* in the figure). This structure is never found in the feet of terrestrial animals, but is found in some climbers such as sloths. Also, the sprawling hind limbs suggest a life of climbing in which pterosaurs hung head up rather than head down. This lifestyle is consistent with an arboreal origin of flight for pterosaurs^{8,11}, an origin well established for bats¹², but still disputed for birds. It is unlikely that pterosaurs had a terrestrial origin of flight as pterosaurs with a narrow wing membrane, clear of

the legs, would have needed a large horizontal tail (not found) to maintain stability in the early evolution of flight.

It seems, then, that when grounded, pterosaurs' stance and gait were more bat-like than bird-like. We should be wary, however, of extending the analogy too far; pterosaur anatomy is very different from that of birds and bats. They appear to have solved the problems of aerial, vertebrate existence in a very different, uniquely pterosaurian way. □

- Wellnhofer, P. *Paläont. Z.* **60**, 329–340 (1986).
- Molnar, R. *Alcheringa* (in the press).
- Wellnhofer, P. *Paleontographica* **149**, 1–30 (1975).
- Wellnhofer, P. *Handbuch der Paläoherpetologie* (ed. Wellnhofer, P.) Part 19 (Fischer, Stuttgart, 1978).
- Wellnhofer, P. *Abh. Bayer. Akad. Wiss.* **141**, 1–133 (1970).
- Padian, K. *Paleobiology* **9**, 218–239 (1983).
- Padian, K. *Postilla* **189**, 1–44 (1983).
- Pennycuik, C. J. *Mem. Calif. Acad. Sci.* **8**, 83–98 (1986).
- Wellnhofer, P. *Ann. Carneg. Mus.* **45**, 5–34 (1974).
- Padian, K. in *Abstr. 3rd Symp. Mesozoic terrest. Ecosyst.* (eds Reif, W. E. & Westphal, F.) 163–168 (Attempto, Tübingen, 1984).
- Wild, R. *Naturwissenschaften* **71**, 1–11 (1984).
- Rayner, J. M. V. in *Biona Report 5 (Bat Flight)* (ed. Nachtigall, W.) 27–74 (Fischer, Stuttgart, 1986).
- Augusta, J. & Burian, Z. *Prehistoric Animals* (Spring Books, London, 1960).
- Wild, R. *Boll. Soc. paleont. ital.* **17**(2), 176–256 (1978).

David M. Unwin is at the Department of Zoology, Reading University, PO Box 228, Reading RG6 2AJ, UK.

Cell cycle control

A cycle is a cycle is a cycle

Andrew W. Murray

In all cells, except those of early embryos, there is co-ordination between cell growth and the discrete steps of the cell cycle that ultimately lead to cell division. This co-ordination occurs at a point in the cell cycle where cells take stock of parameters including the availability of external nutrients and cell size. If the parameters are favourable, the cells enter and become committed to the completion of the mitotic cell cycle. If conditions are unfavourable, the cells either pause, or enter specialized resting states. Cells as diverse as mammalian tissue-culture cells, fission yeast and budding yeast have such commitment points in the G1 portion of the cell cycle (see figure). A paper on page 31 of this issue¹ from Lee and Nurse suggests that the common location of the commitment point reflects a common mechanism that commits cells to progress through the mitotic cell cycle. The authors have identified a human protein that can function in place of the *cdc2* protein, the key regulator of cell cycle commitment in the fission yeast, *Schizosaccharomyces pombe*.

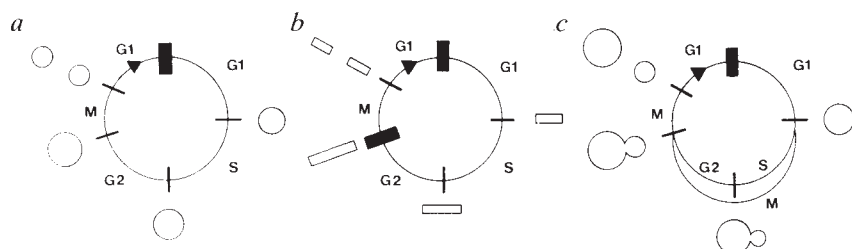
Genetic studies in the budding yeast, *Saccharomyces cerevisiae*, have identified a single commitment point in G1, known as start, and a gene, *CDC28*, whose product is a protein kinase², and whose activity is required to pass start. Until start has been passed, cells in G1 may undergo the

alternative fates of conjugation, entering the meiotic cell cycle, or entering a resting phase called G0. The work of Nurse and his colleagues has shown that the situation in *S. pombe* is more complex, as in addition to start there is a second regulatory point in the cell cycle between G2 and mitosis (reviewed in ref. 3). At this G2/M transition the cell monitors cell size and nutrient availability. If conditions are favourable the cell becomes committed to a rapid entry into mitosis, while if they are unfavourable it enters a G0-like resting state. One gene, *cdc2*, is required for both start and the G2/M transition (see *b* in the figure). The *cdc2* gene product is also a protein kinase, whose activity is high in nutrient-rich media and low when cells are starved⁴, suggesting that the activity of the

cdc2 kinase is required for passage through start. Passage through the G2/M transition requires some additional modification of *cdc2* activity. This conclusion arises from the existence of a class of mutants which interact with *cdc2* at the G2/M transition, but not at start.

What does *cdc2* do at the points where it regulates the cell cycle? One possibility is that it is an intrinsic component of the machinery that carries out the steps of the cell cycle. For instance, at the G2/M transition the activated form of the *cdc2* protein might be maturation promoting factor (MPF), the mitotic inducer that I discussed in a recent News and Views⁵. At start, *cdc2* would have to act in the reactions that lead to the start of DNA synthesis. In this article, I will discuss the alternative view that *cdc2* acts to regulate the intrinsic steps of the cell cycle. In this view, *cdc2* integrates environmental and cell size signals, and when conditions are favourable, allows cells to pass blocks in the cell cycle at start and the G2/M transition.

The similarity between the roles of *cdc2* in *S. pombe* and *CDC28* in *S. cerevisiae* suggested that the proteins were functionally equivalent. This prediction was verified by introducing a library of *S. cerevisiae* genes into an *S. pombe* strain which had a temperature sensitive mutation of the *cdc2* gene and selecting for cells which grew at the high temperature. The only gene⁶ from *S. cerevisiae* that could complement the *cdc2* mutation was *CDC28*. This experiment reinforced previous demonstrations that genes from one species could complement mutations in another species. Lee and Nurse have now extended this type of cross-species cloning to isolate the human homologue of the *cdc2* gene¹. They were able to take advantage of a library of human cDNAs driven by a promoter that works in *S. pombe*. From this library they recovered a single gene that could complement a temperature sensitive mutation in the *cdc2* gene. They went on to establish that the protein product of this gene is present in a variety of mammalian cell lines and that the sequence of this protein is highly homologous to that of the *S. pombe cdc2* gene and the *S. cerevisiae CDC28* gene. Another protein related to *cdc2* has been isolated from a library of human cDNAs by screening it



The cell cycle of: *a*, mammalian tissue-culture cells; *b*, the fission yeast, *Schizosaccharomyces pombe*; *c*, the budding yeast *Saccharomyces cerevisiae*. In all cases the cycle runs clockwise, and the form of the cells at various points is indicated. ■ indicates points of commitment in the cell cycle where homologues of *cdc2* act to regulate progression of the cell cycle.

with a DNA sequence that is strongly conserved in many different protein kinase genes⁷. Although this gene is not identical to the one found by Lee and Nurse, it too is strikingly homologous to *cdc2*. It will be interesting to know if the mammalian *cdc2* homologues are protein kinases, if their activities are regulated by growth factors that control passage through the mammalian cell cycle and what the substrates of these putative kinases are.

The existence of human homologues of the *cdc2* gene suggests that the regulatory mechanisms and molecules of the eukaryotic cell cycle are highly conserved. This argument strongly suggests that the *cdc2* homologues will act at the G1 commitment point in mammalian cells (often known as the restriction point) that is the equivalent of start in yeasts (see figure). Will these proteins also act at a G2/M transition point? The observation that the portion of G1 before the restriction point is the only part of the mammalian cell cycle that is sensitive to the absence of growth factors or partial inhibition of protein synthesis⁸, suggests that there is no growth dependent regulation of the G2/M transition in mammalian cells. The consensus is that the same conclusion is also true for budding yeast. Interestingly, *S. pombe* cells carrying the *CDC28* gene performed the G2 transition precociously⁶ suggesting that the structure of the *CDC28* gene product mimics the secondary modification of *cdc2* required at the G2/M transition in fission yeast.

It is tempting to speculate that some of the differences in cell-cycle regulation reflect differences in growth styles. Mammalian cells lack a rigid cell wall and can divide by cytokinesis (*a* in the figure). They make a single decision in G1 about whether cell size and nutrient levels are sufficient to permit ultimate division and then commit to an uninterrupted mitotic cell cycle. After commitment there is an orderly succession of distinct, cell-cycle phases, late G1, S, G2, and M. Yeasts have a rigid cell wall that constrains the way in which they grow and divide. In budding yeast the solution adopted is to start cell division early in the cycle (*c* in the figure). Bud emergence, the initiation of DNA synthesis and the formation of the mitotic spindle all occur at the end of G1, so that the cell cycle lacks the temporally separated phases of mammalian cells. (The logic of the cycle is similar to that of mammalian cells because the completion of mitosis requires the completion of DNA synthesis and G2 events.) In this scheme the amount of material required to complete cell division, that is the septum between the mother and daughter cell, is made as small as possible. If the cell is in mitosis for a large fraction of the cell cycle, the observed absence of mitotic chromosome condensation may be explained by the inability to transcribe or

replicate condensed chromosomes. In fission yeasts growth is localized so that the cell grows asymmetrically and at the time of cell division a large area of new wall must be synthesized. This strategy allows the cell to maintain the temporally distinct phases of the cell cycle, but implies a large biosynthetic requirement at the end of the cell cycle and presumably a more careful control of cell shape than for cells which grow isometrically. This may explain why fission yeasts have a size and nutrient regulated G2/M transition. Because the conditions that must be met for this transition include events like the completion of DNA synthesis, which are not required for start, the cell cannot use exactly the

same activity to control passage through start and the G2/M transition. *S. pombe* appears to have solved this problem by requiring that an additional modification of *cdc2* be produced in order to make the G2/M transition. □

1. Lee, M.G. & Nurse, P. *Nature* **327**, 31-35 (1987).
2. Reed, S., Hadwinger, J. & Lorincz, A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4055-4059 (1985).
3. Hayles, J. & Nurse, P. *J. Cell. Sci. suppl.* **4**, 155-170 (1986).
4. Sinanis, V. & Nurse, P. *Cell* **45**, 261-268 (1986).
5. Murray, A.W. *Nature* **326**, 542-543 (1987).
6. Beach, D., Durkacz, B. & Nurse, P. *Nature* **300**, 706-709 (1982).
7. Hanks, S.K. *Proc. natn. Acad. Sci. U.S.A.* **84**, 388-392 (1987).
8. Zetterberg, A. & Larsson, O. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5365-5369 (1985).

Andrew W. Murray is in the Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143, USA.

Natural selection

More evolution of cooperation

Robert M. May

I OFTEN leave university committees and discussions with a renewed appreciation for one of the central problems of evolutionary biology: how can natural selection favour cooperative acts within groups, given that selfish individuals can gain by cheating? The problem is made plain by the metaphorical Prisoner's Dilemma, in which each of two individuals chooses either to cooperate or to defect in an encounter. There is a growing amount of work using this metaphor, the most recent of which is reported by Boyd and Lorberbaum on page 58 of this issue¹.

In the Prisoner's Dilemma, if both individuals cooperate, each receives a payoff *R*; if both defect, each receives a lesser amount *P*; and if one chooses to cooperate while the other defects, the nasty defector receives the largest payoff in the game, *T*, while the virtuous cooperator receives the smallest, *S* (that is, $T > R > P > S$). It is immediately clear that, for an isolated encounter, defection is the only rational choice: if the other player cooperates, you receive *T*, which exceeds the *R* you would have gained by cooperating; if the other player defects, you receive *P*, which again exceeds the *S* you would have gained had you cooperated. The paradox arises because if both players follow this reasoning they each gain *P*, whereas each could have gained the larger payoff *R* had they both chosen to cooperate. (In its standard form, the metaphor adds the constraint $R > (S + T)/2$, so that each player gains more by agreeing to sustained cooperation, *R*, than by agreeing to alternate cooperation and defection, out-of-phase, $(S + T)/2$. Either of these two agreements addresses the general problem of cooperation, but it seems sensible to keep the metaphor simple by confining attention to the non-alternating case by requiring $R > (S + T)/2$.)

Axelrod and Hamilton have stimulated recent empirical and theoretical work by showing that, in sequences of encounters between two players, cooperative (nice) strategies can be best, provided the probability, *w*, for the players to meet again is sufficiently high and provided the nice strategy is also provokable (punishing defection) but forgiving (returning to cooperativity when the opponent does)². The simplest exemplar of such a nice, provokable and forgiving strategy is Axelrod and Hamilton's TIT FOR TAT, henceforth called TFT. TFT cooperates on the first move and thereafter repeats the opponent's previous move, thus punishing defection but resuming cooperation once the opponent does. The strategy TFT is a member of a more general class studied by Smale^{3,4}, and has emerged the winner from two computer tournaments organized by Axelrod to explore the relative merits of different strategic approaches to the Prisoner's Dilemma.

How large must the probability for future encounters, *w*, be for TFT to defend itself against invasion by strategies of pure defection (called ALLD, for always defect)? It is helpful to give the answer in some detail, because it illuminates the later discussion. First, the expected gain to either TFT strategist over the course of encounters between them is $R(1 + w + w^2 + \dots) = R/(1 - w)$; this corresponds to *R* for the original encounter, plus *Rw* for the second encounter (which occurs with probability *w*), and so on. Suppose now an ALLD individual appears in the population of TFT players. In a sequence of encounters with any one TFT individual, ALLD will gain the relatively large payoff *T* in the first encounter, but thereafter will find TFT retaliating with defection to yield *P* in all subsequent encounters, for

an expected total payoff $T + Pw/(1 - w)$. If this quantity is less than $R/(1 - w)$, the gain to ALLD will be less than that enjoyed within the TFT population. Thus, TFT is stable against ALLD cheaters provided $w > (T - R)/(T - P)$.

There have been some attempts to test Axelrod and Hamilton's ideas by observing apparent patterns of cooperation among animals in nature. Lombardo has shown, for instance, that parent and non-breeding tree swallows seem to resolve their conflicting interests by conforming roughly to TFT⁵. Lombardo simulated acts of defection by non-breeders who were "helping at the nest", by making it seem as though they had killed nestlings. Parents responded by initial hostility to non-breeders, but soon resumed the cooperative relationship. The difficulty is that precise replication and control is difficult in natural settings.

Possibly the most beautiful empirical test of these ideas is Milinski's laboratory experiment on sticklebacks, reported last month in *Nature*⁶. This experiment is based on the observation that, during the early stages of an attack by a stalking pike, some minnows or three-spined sticklebacks leave the shoal to approach within 4–6 body lengths of the predator for what has been called a "predator inspection visit"⁷. In such visits — where the increased risk of predation is presumably offset by increased knowledge about the predator's identity and current motivational state⁸ — sticklebacks and minnows approach the predatory pike or cichlid more closely when in a group of two than when alone. Two fish engaged in such inspection behaviour can be regarded as cooperating when they either stay close together or take turns in leading the advance toward the predator, whereas if one fish consistently lags behind it may be regarded as a defector (gaining the benefits of inspection with less accompanying risk). Milinski used an ingenious system of mirrors to provide single, three-spined sticklebacks with the illusion of a cooperating companion, or alternatively a defecting companion, as they approached a live cichlid predator. The fish conformed to TFT in consistently approaching the cichlid significantly more closely with a cooperating companion than with a defecting one. If, as I think likely, laboratory tests of TFT and co-operation become a growth industry, researchers must guard against studying phenomena that are so artificial as to be of dubious relevance to the evolution of cooperation in the wild. Among the many nice features of Milinski's experiment is the fact that it is solidly based on the natural history of stickleback behaviour.

There have also been several important developments on the theoretical side. In their report in this issue¹, Boyd and Lorberbaum show that no single strategy

(such as TFT) can be resistant to all kinds of invading strategies for the Prisoner's Dilemma; that is, no single strategy can be 'evolutionarily stable' in this game. Boyd and Lorberbaum argue that their observation "casts doubt on several of Axelrod's conclusions about the evolution of reciprocity". The gist of their analysis can be appreciated by considering the success of TFT against a somewhat less provokable strategy (TIT FOR TWO TATS or TFTT, which does not retaliate until there have been two successive defections), in the presence of a third, less-nice strategy (SUSPICIOUS TIT FOR TAT or STFT, which defects on the first encounter but thereafter plays TFT). Clearly TFT and TFTT strategies always cooperate when playing against each other or against a fellow strategist, and therefore cannot be distinguished in the absence of a third strategy. Against STFT, however, TFT locks into a pattern of reciprocating co-operation and defection on successive encounters, whereas the less provokable TFTT forgives the initial defection by STFT to give a subsequent pattern of mutual cooperation. Thus, TFTT has the edge over TFT if mutant STFT individuals are present, providing a clear example where TFT is not evolutionary stable. Boyd and Lorberbaum provide a more general analysis, and other examples.

I think Boyd and Lorberbaum's paper raises important questions. I suspect, however, that Axelrod and Hamilton's analysis in general, and TFT in particular, may yet turn out to be more robust than Boyd and Lorberbaum fear. For one thing, the TFT/TFTT/STFT example (and other examples discussed by the authors) depends to a large extent on the strategies being rigidly adhered to. In the real world, on the other hand, there is much less precision; even in Milinski's relatively exact experimental situation, not all co-operation is rewarded and not all defection punished. If TFT is played somewhat probabilistically, punishing defection with a probability p less than 100 per cent, then TFT and STFT will eventually settle to mutual cooperation. TFTT will still enjoy an advantage in this circumstance, but the advantage — even for p close to unity — can be small if w is large enough. In other words, I think the interesting issues raised by Boyd and Lorberbaum should be explored using strategies with some realistic stochasticity in them, which could weaken some conclusions that depend on the first move determining the entire future pattern of interaction.

A second, probably more significant, suggestion is that we should examine the relative success of TFT and, say, TFTT in the presence of some representative ensemble of other mutant strategies (rather than in the presence of only one, such as STFT). This admittedly vague suggestion is motivated by the observation that TFTT

has a gross vulnerability of a kind that TFT does not. If I know you are playing TFTT, I simply alternate defection and co-operation: on alternate turns I gain T and R , while your excessive niceness limits you to S and R . There are obvious problems in deciding how to generate an appropriate ensemble of mutant strategies (which is why this a News and Views article and not a research paper), but I guess TFT may well regain its evolutionary stable status in such an analysis.

Two other refinements of Axelrod and Hamilton's ideas deserve mention. In their original paper and in the computer tournaments, all payoffs had the same intrinsic value; future payoffs were discounted only in proportion to the probability of their occurrence. At that time, I observed in a News and Views article⁴ that various effects could result in future gains being worth less than present ones, which would effectively mean the parameter w measures the probability of future encounters multiplied by a discount factor to allow for the lower value of such future payoffs. Milinski observes, however, that future gains may on occasion be worth more than present ones⁶. In his stickleback–cichlid experiment, for example, the payoff is information, and this payoff becomes larger as the predator is approached more closely toward the end of the sequence of cooperative acts between two sticklebacks. Indeed, Milinski argues that this fact is likely to make w relatively large for his system, thus making it particularly suitable for experimental study. More generally, we can envisage other situations where early encounters are used to build trust within a group, so that eventually it may undertake an enterprise — rob a bank — with a large reward. These complications are commonsensical and do not involve fancy mathematics; they nevertheless seem to me to deserve more systematic attention than they have so far received.

The usual models assume the probability for a future encounter, whether discounted or not, is the same for all pairs. Feldman (as reported by Packer⁹) has looked at what happens when the probability of a future encounter depends on past outcomes, which is a complication likely to arise in practice. One way to deal with cheaters is not to deal with them again. In particular, Feldman has studied the situation in which the probability, w , of having a further encounter with a cooperator is greater than the probability, u , of having a further encounter with a defector. Such a situation obviously favours the evolution of TFT over defecting strategies. Less obviously, Feldman finds certain values of w and u can lead to polymorphisms, in which TFT and ALLD co-occur at equilibrium. Such a situation was not envisaged in the original analysis by Axelrod and Hamilton.

In short, there is much scope for further experimental and theoretical work on TFT and the metaphor of the Prisoner's Dilemma. My belief is that the theory needs to take more account of intrinsic stochasticities and of evolutionary stability against representative ensembles of mutant strategies, whereas experimental studies will be best if based on a sound appreciation of the natural history of the particular cooperative phenomenon under investigation. □

1. Boyd, R. & Lorberbaum, J.P. *Nature* **327**, 58-59 (1987).
2. Axelrod, R. & Hamilton, W.D. *Science* **211**, 1390-1396 (1981).
3. Smale, S. *Econometrica* **48**, 1617-1634 (1980).
4. May, R.M. *Nature* **292**, 291-292 (1981).
5. Lombardo, M.P. *Science* **227**, 1363-1365 (1985).
6. Milinski, M. *Nature* **325**, 433-435 (1987).
7. Pitcher, T.J., Green, D.A. & Magurran, A.E. *J. Fish Biol.* **28**, 439-448 (1986).
8. Pitcher, T.J. in *The Behaviour of Teleost Fishes* (ed. Pitcher, T.J.) 294-337 (Croom Helm, London, 1986).
9. Packer, C. *TREE* **1**, 142-143 (1986).

Robert M. May is Class of 1877 Professor of Zoology at Princeton University, Princeton, New Jersey 08544, USA.

Protein transport

Signals and salvage sequences

Graham Warren

PROTEINS that leave the cytoplasm during or after synthesis must contain a signal, a stretch of amino acids that binds to the correct compartment and enables the rest of the protein to cross into it. Each compartment is recognized by a characteristic signal (see figure) which acts autonomously as it can usually be transferred experimentally to any cytoplasmic protein, thereby making it enter the appropriate compartment. These signals must have evolved very early in evolution and recent work exploiting the genetics of yeast suggests two possible mechanisms. The first involves spontaneous mutations in the amino terminus of the protein, the region that usually mediates transfer across both endoplasmic reticulum (ER) and mitochondrial membranes; and the second involves DNA transposition which would introduce an entire signal into the gene encoding the protein to be transported.

The first of these two mechanisms has been demonstrated by Vassarotti and colleagues¹, who took a yeast mitochondrial protein (the β -subunit of F₁-ATPase) and deleted the pre-sequence so that the protein was now expressed in the cytoplasm. By expressing it in yeast lacking the wild-type gene and looking for growth on glycerol, these authors identified pseudorevertants that could once more transport the protein into mitochondria. These revertants were found at a frequency of 1 in 10⁷ and almost all of the point mutations involved exchanging acidic amino acids for neutral or basic ones, thereby changing the amino terminus into a more basic amphiphilic helix. In other words, the mutations tended to make the amino terminus look more like a mitochondrial pre-sequence (see figure).

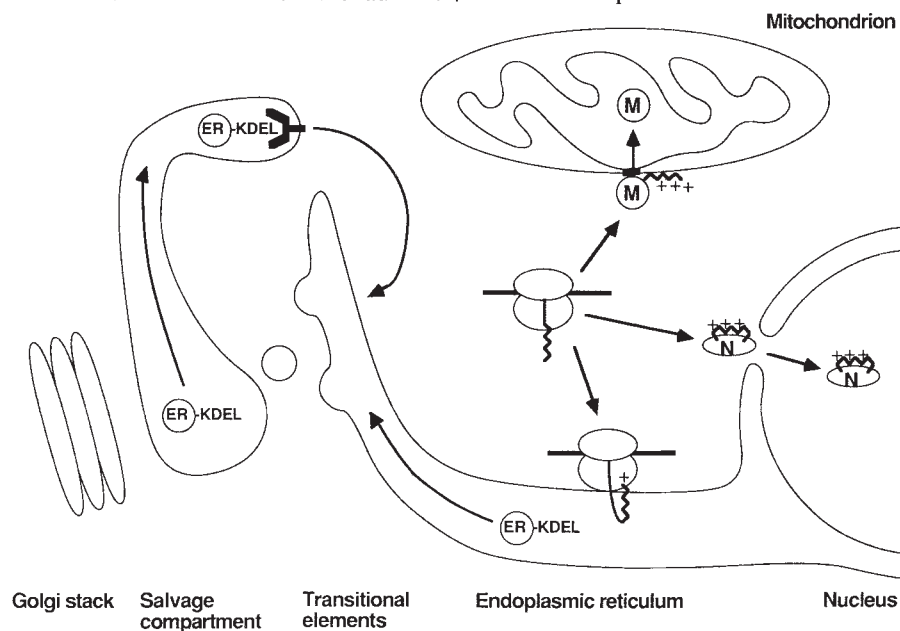
Evidence implicating the second proposed mechanism comes from Baker and Schatz², who used recombinant DNA technology to mimic the transposition process. They took another mitochondrial protein (subunit IV of cytochrome *c* oxidase), replaced the pre-sequence with

random DNA fragments from *Escherichia coli*, and asked whether any of these fragments could act as import signals. Those that could were sequenced and found to resemble authentic sequences showing a marked bias against acidic amino acids and a high proportion of positively charged ones. But the real surprise was that so many were found to function in this way. Between 2 and 3 per cent of the fragments were functional and when the authors

used DNA fragments from a mammalian cytosolic protein, dihydrofolate reductase (DHFR), the amount rose to 5 per cent.

Kaiser *et al.*³ have taken the same approach as Baker and Schatz but have looked instead at transfer into the ER which also requires an amino-terminal signal but with different consensus features⁴. They replaced the signal at the amino terminus of yeast invertase with random DNA fragments from human genomic DNA and looked for mutants that grew on sucrose and were therefore secreting invertase. After correcting for those fragments that would contain stop codons or be in the wrong reading frame, they found that around 20 per cent of DNA fragments could function as signal sequences. Functional sequences were enriched for hydrophobic amino acids and depleted in charged amino acids.

What do these data tell us about ER signal sequences and mitochondrial pre-sequences? Obviously the term 'sequence' is a misnomer because sequences are not conserved between signals. This fact has long been realized and is further emphasized by the recent work. Rather, the signal is a domain which can be formed by many different sequences. Present-day consensus sequences⁴⁻⁶ are the result of a



Major routes taken by proteins that leave the cytoplasm in eukaryotes (other than plants). Protein synthesis begins in the cytoplasm and, for nuclear and mitochondrial proteins, is completed before transfer begins. Nuclear proteins have a short, basic sequence within the protein that ensures delivery to the nuclear matrix and retention within⁵. Most mitochondrial proteins have a longer, amphiphilic sequence at the amino terminus that is rich in basic and hydroxylated amino acids but poor in acidic ones⁶. Proteins are usually directed into the matrix unless additional sequences interrupt transport and they probably cross both mitochondrial membranes at adhesion sites⁸. Most proteins destined for the ER contain a basic amino terminus followed by a stretch of hydrophobic amino acids⁴. As this emerges from the ribosome, it interacts with signal-recognition particle and further synthesis is delayed until the complex reaches the docking protein on the ER membrane²⁰. Release of the block is followed by transfer, usually coupled to translation, and the signal is usually cleaved. Most proteins are exported from the ER but those soluble proteins that function in the lumen of the ER have a carboxy-terminal tetrapeptide (KDEL = Lys-Asp-Glu-Leu) which may serve to recover protein lost by inadvertent export¹¹. Recovery would require a specific receptor which might act at a compartment between the transitional elements of the ER and the Golgi stack^{15,16} and could be termed the salvage compartment.

long evolutionary process and the new work shows that sequences breaking these rules still work. Of course they work very inefficiently but this must have been important early in evolution when the cytoplasm surrounded itself with an impermeable lipid bilayer and many proteins had to be inserted to provide the vital exchange of material with the environment. The presence of so many potential signals must have allowed cells then, as perhaps now, to try out different proteins in other compartments. Those that conferred a selective advantage would continue to be transported, the signals mutating further to increase the efficiency of transfer.

What this idea does not explain is why these signals do not normally function *in vivo*. If up to 20 per cent of cellular DNA can encode functional signals, why are proteins not being misdirected all the time? The simple answer comes from work by Hurt and Schatz⁷ who found that residues 26–49 of DHFR can serve as a mitochondrial import signal when attached to the amino terminus of the native DHFR protein. The reason why it does not function in the native protein is clear from the crystal structure. The hydrophobic face of the 'signal' is directed towards the interior of the protein and is therefore not available for interaction with the mitochondrial membrane. When attached to the amino terminus of DHFR it exists as an exposed domain that can be cleaved by proteases. Clearly, signal domains not only have certain consensus features, they must also be exposed if they are to function.

What happens to these proteins once they have been delivered to the appropriate compartment? Nuclear proteins have only to cross the nuclear pore to reach their destination. The signal is needed to keep the protein there and so is not cleaved⁸. Mitochondrial pre-sequences direct the protein to the matrix unless they contain additional information to stop them at an intermediate point⁸. The pre-sequence is usually cleaved. ER proteins end up as either membrane or soluble proteins, the latter including secretory and lysosomal proteins which must then be exported from the ER. Receptors have been postulated to carry them to the next compartment⁹ but none has been found; the fact that oocytes will act as surrogate for any secretory protein, including bacterial ones¹⁰, receptors for which are unlikely, argues for bulk movement of these proteins from the ER.

But how do the proteins that function in the lumen of the ER resist export? Munro and Pelham¹¹ have now compared the sequence of three major ER proteins, protein disulphide isomerase (PDI) and two glucose-regulated proteins, grp78 and grp94. They found that all these proteins have the same tetrapeptide (Lys-Asp-

Glu-Leu) at the carboxy terminus. When this sequence is removed from grp78, the truncated protein is slowly secreted. When it is extended by just two amino acids it is again secreted. The critical experiment was to transfer the sequence to the carboxy terminus of lysozyme, a secretory protein. The protein is then no longer secreted but remains in the ER. This important result represents the only signal other than that for lysosomal enzymes¹² that is known to function during intracellular transport.

How would such a sequence work? It seems unlikely that there is an anchor in the ER membrane because these sequences are found on major ER proteins and there is no candidate present in sufficient quantity to bind them all. Furthermore, sevenfold over-expression of grp78 does not result in secretion¹¹ and fractionation experiments show that it is rapidly released on breaking the ER¹³. *In vivo*, grp78 must be bound to some extent to the ER membrane to explain the slow rate at which the truncated protein is exported. This suggests that the most likely explanation¹¹ is a receptor recognizing the tetrapeptide only after the proteins have been inadvertently exported from the ER. Only small amounts of the receptor would then be needed, cycling between the ER and the compartments to which the proteins have been inadvertently sent. This would amount to a salvage operation and the tetrapeptide could rightly be described as a salvage sequence. Secretory and lysosomal proteins leave the ER in vesicles which bud from the transitional elements and fuse with the *cis*-most cisterna of the Golgi stack. This cisterna contains the enzyme mannosidase I¹² which continues the trimming of mannose residues started by ER mannosidase and yields oligosaccharides containing five mannose residues. If the soluble ER proteins are continuously retrieved from this *cis* Golgi cisterna then they should bear oligosaccharides containing five mannose residues. Only one of the three soluble ER proteins, grp94, is known to contain oligosaccharides, and this protein is the same as ERp99 (ref. 11) which has oligosaccharides containing six or more mannose residues but not five (ref. 14). So from where are the proteins recovered if not from the *cis* Golgi cisterna? Fortunately, there seems to be an intermediate compartment between the transitional elements of the ER and the Golgi stack in which viral proteins accumulate at low temperature¹⁵ and into which some viruses bud¹⁶. The physiological function of this compartment is unknown but if ER proteins are recovered from it then it could be called the salvage compartment, in which the receptor would bind the tetrapeptide (see figure) and deliver the protein back to the ER where a difference in environment, perhaps of pH (as, for example,

in endosomes), would cause release followed by receptor recycling. All this assumes that the tetrapeptide is available for binding, which is reasonable given the charge and location of the tetrapeptide. The carboxy terminus should be the last part of the protein to fold, which should ensure exposure on the protein surface.

If a salvage mechanism exists, then the sorting of soluble proteins at the exit site of the ER must be inefficient compared with the sorting of ER membrane proteins¹⁷. The difficulty is illustrated by receptor-mediated endocytosis in which there is a mechanism for concentrating receptors many thousand-fold over the rest of the plasma membrane¹⁸, but none for preventing uptake of proteins added in the fluid phase¹⁹. Fluid uptake can only be minimized by generating the vesicles with a high rate of surface area to volume. Receptor-mediated endocytosis can therefore sort membrane proteins (such as receptors) better than soluble ones. The problem at the level of the ER is worse because so many soluble proteins have to be exported, particularly in secretory cells. A high volume of export makes it even more difficult to keep back soluble ER proteins, so a salvage mechanism may be the only solution.

All this speculation assumes that there are proteins that function in the lumen of the ER and that they would not be able to function if they were permanently anchored to the ER membrane. PDI is thought to construct disulphide bridges in newly synthesized secretory proteins and grp78, a heat-shock cognate that is also identical to immunoglobulin heavy-chain binding protein¹³, is thought to be involved in the assembly of secretory proteins. Pelham argues that grp78 belongs to a class of proteins whose role is to assemble proteins or repair damaged or incorrectly folded ones. If soluble ER proteins are to carry out their function, they may well have to reach parts of secretory proteins that anchored proteins could not reach. □

1. Vassarotti, A. *et al.* *EMBO J.* (in the press).
2. Baker, A. & Schatz, G. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
3. Kaiser, C. A. *et al.* *Science* **235**, 312–317 (1987).
4. von Heijne, G. *J. molec. Biol.* **184**, 99–105 (1985).
5. Dingwall, C. & Laskey, R. A. *Rev. Cell Biol.* **2**, 367–390 (1986).
6. von Heijne, G. *EMBO J.* **5**, 1335–1342 (1986).
7. Hurt, E. C. & Schatz, G. *Nature* **325**, 499–503 (1987).
8. Colman, A. & Robinson, C. *Cell* **46**, 321–322 (1986).
9. Lodish, H. F. *et al.* *Nature* **304**, 80–83 (1983).
10. Wiedmann, M. *et al.* *Nature* **309**, 637–639 (1984).
11. Munro, S. & Pelham, H. *Cell* **48**, 899–907 (1987).
12. Kornfeld, R. & Kornfeld, S. *Rev. Biochem.* **54**, 631–664 (1985).
13. Bole, D. G. *et al.* *J. Cell Biol.* **102**, 1558–1566 (1986).
14. Lewis, M. J. *et al.* *J. biol. Chem.* **260**, 6926–6931 (1985).
15. Saraste, J. & Kuismanen, E. *Cell* **38**, 535–549 (1984).
16. Toozé, J. *et al.* *Eur. J. Cell Biol.* **33**, 281–293 (1984).
17. Brands, R. *et al.* *J. Cell Biol.* **101**, 1724–1732 (1985).
18. Bretscher, M. S. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **77**, 4156–4159 (1980).
19. Marsh, M. & Helenius, A. *J. molec. Biol.* **142**, 439–454 (1980).
20. Hortsch, M. & Meyer, D. I. *Int. Rev. Cytol.* **102**, 215–242 (1986).

Graham Warren is Professor of Biochemistry, University of Dundee, Dundee DD1 4HN, UK.

Quasicrystals

Amorphous, crystalline or both?

M. Widom

THE structure of icosahedral quasicrystals¹, discussed in a News and Views article² last month, remains a mystery and a source of controversy. Two leading models, the quasiperiodic-crystal model of Levine and Steinhardt³ and the icosahedral-glass model of Stephens and Goldman⁴, both disagree with details of the experimental findings. At a recent meeting*, proposals were made for modifying each model to obtain agreement with details of the X-ray diffraction pattern. Various experimental and theoretical results suggest that the truth combines elements of both models. Quasicrystals may be a unifying link between crystalline and amorphous metals.

Quasicrystals, discovered by D. Shechtman (Israel Institute of Technology), are metallic alloys whose electron-diffraction patterns combine sharp, crystallographic peaks with non-crystallographic, icosahedral symmetry. Efforts to explain the structures by twinning⁵ fail to account for the observed golden-mean ratios between peak positions (A. R. Kortan, AT&T Bell Labs) and need double diffraction to explain the peaks observed in X-ray diffraction patterns (P.A. Bancel, University of Pennsylvania). The quasiperiodic-crystal and icosahedral-glass models overcome these flaws.

The quasiperiodic-crystal model³ uses a three-dimensional generalization of the Penrose pattern to produce a structure that combines long-range, icosahedral bond-orientational order with translational quasiperiodicity. The icosahedral-glass model⁴, in contrast, packs oriented icosahedra joined at random on their faces. The peak positions for both models are in excellent agreement with X-ray diffraction experiments. The peak intensities agree qualitatively with experiments in that the intensities fall off at large values of the perpendicular momentum. (Perpendicular momentum measures mismatch between the quasiperiodicity of the quasicrystal and the periodicity of the X-rays.) Both models fail to explain properly the observed widths of the X-ray diffraction peaks, which are slightly broadened, their width proportional to the perpendicular momentum (P.M. Horn, IBM, Yorktown Heights). The icosahedral-glass model produces broadened peaks, but the widths are proportional to a higher power of the perpendicular momentum (A.I. Goldman, Brookhaven National Lab). Thus, the two models straddle the true behaviour of the peak widths.

The quasiperiodic-crystal can be modified by the inclusion of 'phasons', which are like phonons but carry perpendicular momentum instead of ordinary momentum. These excitations correspond to rearrangements of atoms that destroy the quasiperiodicity but do not otherwise distort the structure. The inclusion of random, phason excitations leads to the correct behaviour of the peak widths^{6,7} and also predicts unusual peak shapes (J.E.S. Socolar and D.C. Wright, University of Pennsylvania; D.R. Nelson and M.V. Jaric, Harvard University) which can be observed experimentally (J.D. Budal, Oak Ridge Labs; D.M. Follstaedt and J.A. Knapp, Sandia Labs). Modifying the icosahedral-glass model is harder. The original model puts new icosahedra indiscriminately on any face of the growing cluster where they can fit. Invariably, these clusters contain many cracks that cannot be filled with atoms in their optimal environments. More careful growth modelling that reduces the number of cracks, and the introduction of higher-order positional correlations, are unable to reproduce the observed linear dependence of peak width on perpendicular momentum.

Intrinsic disorder provides circumstantial evidence favouring models such as the icosahedral glass. The peak widths were first observed in splat-cooled AlMn in which the grain size is typically a micrometre⁸. Recently, quasicrystals of AlLiCu have been produced with slower cooling rates achieving grain sizes of centimetres and showing beautiful faceting. Curiously, the peak widths in the supposedly high-quality AlLiCu quasicrystals were comparable to those in the splat-cooled AlMn quasicrystals. Horn suggested this shows a source of intrinsic disorder in quasicrystals on a scale of hundreds of angstroms.

S.C. Moss (University of Houston) warned against drawing strong conclusions based on the assumption that the AlLiCu samples are the most ordered possible quasicrystal. The actual rate of quasicrystal growth in slowly cooled AlLiCu is comparable to that in the splat-cooled AlMn because the grains of AlLiCu are so large. Thus it is not surprising that both have similar degrees of disorder. The beautiful facets on the AlLiCu quasicrystals may also be misleading. J. Toner (IBM, Yorktown Heights) said that even highly defected crystals may have facets which appear macroscopically flat. The issue will not be decided experimentally until defects are removed from the AlLiCu samples. In

fact, ion-channelling experiments in carefully grown quasicrystals (M.A. Marcus, AT&T Bell Labs) indicate translational order on a scale of micrometres.

There are already theoretical models that bridge the gap between the perfect, quasiperiodic crystal and the icosahedral glass. K.J. Strandburg and I presented Monte Carlo simulations of a binary mixture of Lennard-Jones atoms in two dimensions. The mixture spontaneously forms a quasicrystalline equilibrium state at low temperatures⁹. Thermodynamic stability depends on the configurational entropy produced by randomly rearranging the tiles of the Penrose pattern. The structure is thus equivalent to the perfect, quasiperiodic crystal, modified by the inclusion of phasons. C.L. Henley (Cornell University) described models of three-dimensional quasicrystals that also allow local deviations from perfect quasiperiodicity. In these models the diffraction spots are delta functions (Henley) with power-law, diffuse background (Nelson, Jaric).

An exciting view of quasicrystals emerged from many of the experimental talks. This places quasicrystals between crystals and amorphous metals, sharing features of both. Quasicrystals possess sharp diffraction peaks, and there is also strong evidence for crystalline short range order. X-ray absorption fine structure studies of AlLiCu quasicrystals (Y. Ma and E.A. Stern, University of Washington) find that the chemical environment of the copper atoms are virtually identical to those of a crystalline phase of similar composition. Comparison of the Patterson functions of crystalline and quasicrystalline phases of AlMnSi inspired J.W. Cahn (NBS) to remark that the local environments are extraordinarily similar. Glassy behaviour in quasicrystals was found by observing tunnelling systems (N.O. Birge, AT&T Bell Labs). Finally, Moss demonstrated structural similarities between icosahedral quasicrystals and amorphous metals. He compared the size-broadened, quasicrystalline AlMn structure function with the glassy phase of the same composition and remarked "It is as if glasses are microquasicrystalline". □

1. Shechtman, D., Blech, I., Gratias, D. & Cahn, J.W. *Phys. Rev. Lett.* **53**, 1951-1953 (1984).

2. Guyot, P. *Nature* **326**, 640-641 (1987).

3. Levine, D. & Steinhardt, P.J. *Phys. Rev. Lett.* **53**, 2477-2481 (1984).

4. Stephens, P.W. & Goldman, A.I. *Phys. Rev. Lett.* **56**, 1168-1171 (1986).

5. Pauling, L. *Nature* **317**, 512-514 (1985); *Phys. Res. Lett.* **58**, 365-368 (1987).

6. Lubensky, T.C., Socolar, J.E.S., Steinhardt, P.J., Bancel, P.A. & Heiney, A. *Phys. Rev. Lett.* **57**, 1440-1443 (1986).

7. Horn, P.M., Malzfeldt, W., DiVincenzo, D.P., Toner, J. & Gambino, R. *Phys. Rev. Lett.* **57**, 1444-1447 (1986).

8. Bancel, P.A., Heiney, P.A., Stephens, P.W., Goldman, A.I. & Horn, P.M. *Phys. Rev. Lett.* **54**, 2422-2425 (1985).

9. Widom, M., Strandberg, K.J. & Swendsen, R.H. *Phys. Rev. Lett.* **58**, 706-709 (1987).

* March Meeting of the American Physical Society 16-20 March 1987, New York.

M. Widom is at the Department of Physics, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213, USA.

Unusual codon usage of HIV

SIR—It is interesting that although codon usage bias is organism-specific or gene-specific as far as the third degenerate position is concerned^{1,2}, it is almost general in the first two non-degenerate codon positions³. Human immunodeficiency virus (HIV), the aetiological agent of AIDS (acquired immune deficiency syndrome), follows the general pattern in the first two positions but the bias is shifted towards a distinct preference for adenine at the expense of pyrimidine bases, in particular cytosine. The shift is markedly amplified in the third codon position of HIV genes while adenine is rare in the third codon position of genes in most other organisms and eukaryotes in particular. Purines predominate over pyrimidines in all codon positions of all HIV genes, which is unprecedented.

In an analysis of a recent compilation⁴ of codon usage in 1,638 genes we have found no gene that is as deviant from mean codon usage as the genes of HIV. Nonetheless there are a few other viruses (influenza, human papilloma, cauliflower mosaic, papova, visna) showing a similar trend of adenine preference in their genes. Sharp⁵ has found that HIV shares a surprisingly high number of the most preferred codons with influenza and cauliflower mosaic viruses. We add that most of these shared codons contain adenine in the degenerate position.

What accounts for the overrepresentation of adenine in HIV? It cannot be genome type or a factor specific for the host organism because these vary greatly among different adenine-rich viruses. Neither is it an involvement of reverse transcriptase in the viral reproduction cycle because the Moloney murine leukaemia retrovirus, for example, does not show a preference for adenine. Although HIV is not extreme in its A+T content (37 per cent A + 21 per cent T) we examined codon usage in A+T rich human genes and found correspondingly increased amounts of adenine in the third position of their codons. But the amount of adenine is far less than in HIV genes.

We have also considered the possibility that the excessive adenine is related to the great genetic variability of HIV. In this respect it is of interest that the mutation rate in vertebrate immunoglobulin genes correlates positively with the local A+T content⁶. But the overrepresentation of adenine in HIV is highest in the *pol* gene and lowest in the more variable *env* gene. Also, adenine distribution does not appear to be concentrated in the hyper-variable segments of *env*.

In summary, we are unable to offer a plausible explanation for the existence of

a group of viruses (but no bacteriophage), of which HIV is the most extreme representative, that seem to prefer adenine to alternative bases. Finding an explanation for this peculiar coding strategy could contribute to a better understanding of the evolution and pathogenesis of HIV.

JAROSLAV KYPR

JAN MRÁZEK

*Institute of Biophysics,
Czechoslovak Academy of Sciences,
612 65 Brno, Czechoslovakia*

1. Grantham, R., Perrin, P. & Mouchiroud, D. *Oxford Surv. Evol. Biol.* 3, 48-81 (1986).
2. Ikemura, T. *Molec. evol. Biol.* 2, 13-34 (1985).
3. Kypr, J. & Mrázek, J. *Int. J. biol. Macromol.* 9, 49-53 (1987).
4. Maruyama, T., Gojoberi, T., Aota, S. & Ikemura, T. *Nucleic Acids Res.* 14, r151-197 (1986).
5. Sharp, P.M. *Nature* 324, 114 (1986).
6. Perrin, P. *Nucleic Acids Res.* 12, 5515-5527 (1984).

How many reactor accidents will there be?

SIR—The argument of Islam and Lindgren¹ that there is some difficulty in reconciling the predictions of future nuclear incidents based on observations of historical accidents with the claims made by nuclear designers that the chance of a nuclear reactor incident is one in 1 million per reactor year is based on the assumption that the incidents can be accurately described by the Poisson distribution involving two parameters—the failure rate, r , and years of reactor operation, T . Edwards² correctly points out that they have unwittingly adopted a bayesian approach and implicitly used a uniform prior probability density for the failure rate. He then proposes estimation based on transforming the likelihood for r to a likelihood for the probability of one or more incidents, $P = P(r) = 1 - e^{-rT}$. The difference between the two analyses is that, in a bayesian framework, Islam and Lindgren use a uniform prior on r , while Edwards implicitly uses a uniform prior on P , which is equally dubious. None of these authors has combined engineering expertise with data derived from operational experience, especially the highly relevant prior information contained in studies on nuclear reactor safety such as the Rasmussen³ report for the Nuclear Regulatory Commission or articles by Rasmussen⁴, Lewis⁵ and Groer⁶.

If one treats r as a random variable, the only coherent way to incorporate the

extensive engineering information is to use the bayesian methodology with a prior distribution based on the best knowledge available at the time. Rasmussen⁴ and Lewis⁵ argue that the logarithm of r (to the base 10) for complete core meltdowns should be normally distributed with mean 4 and variance 1; alternatively, to the base e , this prior probability is $\ln r \sim N(-9.21, 5.30)$. Conditional on r , the density of time to the next incident is exponential with $p(x|r) = r e^{-rx}$; thus the posterior density of the failure rate (or of $P(r)$) can be obtained by direct integration:

$$p(r|x) = C \int p(x|r)p(r|0) dr \\ = C \int r e^{-rx} p(r|0) dr$$

where $p(r|0)$ denotes the prior density at time 0 without operating history and C is a normalizing constant. With the lognormal prior, integration is straightforward but messy; it has been reported, in a slightly different context, by Lewis⁵ and Groer⁶.

Combining information for partial core meltdowns⁶ of PWR-9 with the information for complete core meltdowns⁵, we find by a linear scaling of $\ln r$ that the prior for partial core meltdowns is $\ln r \sim N(-7.82, 5.3)$. This implies that the rate of partial to complete core meltdowns is about 4 to 1. Based on this lognormal prior it can be shown that the probability there is at least one partial core meltdown incident in ten years (374 reactors in operation from 1986 to 1996) is equal to 0.75. Because of the optimistic Rasmussen, Groer and Lewis priors, the estimate is reduced from the 0.86 figure found in Islam and Lindgren. However, it is much better to do this than to use a uniform prior which places equal weight on all values of r . Eventually the data will dominate any prior, but it is still too early to tell.

Insights on how operational experience combines with engineering expertise can be easily demonstrated if one uses, instead of the lognormal, a gamma distribution of the prior probability for which analytical results are available. When the prior density is approximated by a member of the gamma family with parameters (α, β) , expected time to the next incident given $n(T)$ incidents by T is

$$E[X|n] = (T + \beta)[n + \alpha - 1]^{-1} \\ = (T/n)[n/(n + \alpha - 1)] + \\ (\beta/\alpha - 1)[1 - n/(n + \alpha - 1)]$$

provided $n + \alpha > 1$. With $0 \leq n + \alpha \leq 1$, the conditional expectation is infinite. As one might suspect, the expected time to the next incident is a weighted sum of the arithmetic mean T/n and the prior expectation of X . Unfortunately, it is not possible to fit the gamma well (simul-

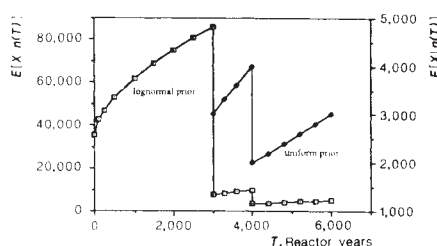


Fig. 1 Expected time to the next incident.

taneously at the tails and the origin) for the lognormal with the above parameters.

In the figure we graph the expected time to the next incident as a function of operating experience; the lognormal prior uses the left-hand scale. For simplicity we assume that the first and second incidents occur at 3,000 and 4,000 reactor years, respectively, and that one calendar year with today's inventory of operating nuclear reactors corresponds to about 400 reactor years as of 1985. At the second incident the conditional expectation decreases to about 3,000 reactor years. Not only is there a decrease following each incident but it should be noted that the slope of each successive line segment decreases over time. This is due, very simply, to the fact that the non-occurrence of an incident in late time periods carries less information than non-occurrence of incidents did in the early lives of nuclear reactor operations. The graph that corresponds to the uniform prior used in the earlier articles by Islam and Lindgren and Edwards makes use of the right-hand scale.

TAT CHI CHOW
ROBERT M. OLIVER

Operations Research Center,
University of California,
Berkeley, California 94720, USA

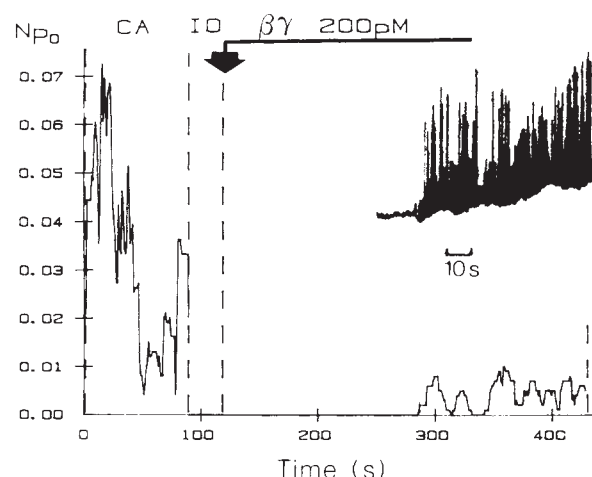
- Islam, S. & Lindgren, K. *Nature* **322**, 691-692 (1986).
- Edwards, A. W. F. *Nature* **324**, 417-418 (1986).
- Rasmussen, N. C. *et al. Reactor Safety Study, Wash-1400 (NUREG 75/014)* (US Nuclear Regulatory Commission, Washington, DC, 1975).
- Rasmussen, N. C. *Ann. New York Acad. Sci.* **365**, 20 (1981).
- Lewis, H. W. *Nucl. Sci. Engng.* **86**, 111-112 (1984).
- Groer, P. C. *Low Probability High-Consequence Risk Analysis* (eds Waller, R. & Covello, V. T.) (Plenum, New York, 1984).

G protein opening of K⁺ channels

SIR—Logothetis *et al.*¹ claim that $\beta\gamma$ -subunits of G proteins acting at nanomolar concentrations are the mediators of the activation of K⁺ channels by muscarinic acetylcholine receptors (mAChR) in the heart. The conclusion is unsettling, as noted in the News and Views article by Bourne², because it raises a difficult question—how does an agonist specifically activate K⁺ channels when it uses free $\beta\gamma$ that can be released from several G proteins? The solution to the quandary turns out to be simple. It is provided by our discovery³ that the G protein, G_k, stimulates mammalian atrial K⁺ channels at picomolar concentrations whereas pre-activated G_k, which has the same $\beta\gamma$ as G_k but differs in its α -subunit, has no effect at concentrations several hundred times greater. Specificity thus comes from the α confirmed by Hescheler *et al.*, who show that the α and not the $\beta\gamma$ of porcine brain G_o regulates neuronal calcium channels⁴.

So why did Logothetis *et al.* find that $\beta\gamma$ are stimulatory and that α is without

Cell-attached (CA) and inside-out (IO) patch activity from a 14-day old chick atrial cell. 200 pM $\beta\gamma$ activates the channel. Channel amplitudes, 2.9 pA.



effect but when mixed with $\beta\gamma$ quenches its effect? The answer does not lie in the electrophysiological data as the particular K⁺ channels involved can be identified unambiguously. It probably lies in the nature of the G protein preparations used to obtain the data and we offer the following explanations.

First, the α was either the wrong one, partially denatured, or subjected to an inadequate activation protocol. Neer *et al.* originally described three substrates for pertussis toxin (PTX) in brain⁵, but only two were tested by Logothetis *et al.* No data were given as to whether the activation protocol used to convert α -GDP complexes to α -GTP γ S complexes actually resulted in nucleotide exchange. Apparently, 500 to 1280 nM α were treated for 30 min at 22 °C with 200 nM guanine nucleotide in the presence of 2 mM Mg²⁺. In our hands this would lead to little if any nucleotide exchange and to some inactivation.

Second, a contaminant, not the approximately 23 nM $\beta\alpha$, may have caused opening of K⁺ channels. The contaminant could be a phospholipase that, on modifying the lipid environment of the channel, causes it to open, or an $\alpha\beta\gamma$ -G protein, such as G_k, that is active in opening K⁺ channels. Phospholipases are known to mimic certain hormonal effects at the plasma membrane⁶. Contamination of the α preparations with a heat labile protease could then account for the quenching of $\beta\gamma$ were the phospholipase or G_k sensitive to the protease and the protease insensitive to the inhibitors that were used. Although the exact details may differ, the point is that contaminants could account for the results.

As to the possible presence of G_k, no attempt to quantify contamination by a PTX-sensitive $\alpha\beta\gamma$ in the $\beta\gamma$ preparations is described, other than a Coomassie blue stain of a gel to which 2 μ g of $\beta\gamma$ had been applied. This method would not allow detection of < 0.1 μ g α ; methods to increase the sensitivity of the assay 100–1,000-fold are available⁵. Although Logothetis *et al.*

state that $\beta\gamma$ prepared by two different procedures was used, citation to only one is given. In it, extracted G proteins are stabilized with fluoride, aluminium chloride and Mg²⁺. For G_s, with which this can be measured, this type of treatment leads to a preactive state—undissociated in the cold yet active in *cyc*[−] reconstitution assays without addition of activating ligands⁷. It is therefore possible that $\alpha\beta\gamma$ complexes contaminating $\beta\gamma$ at ratios between 1 in 10,000 and 1 in 1,000 would be active on K⁺ channels as tested by Logothetis *et al.* We have observed activation of K⁺ channels of the type they obtained with 20 nM $\beta\gamma$ using only 20–100 pM GTP γ S-activated G_k. Furthermore, in our hands 2 nM of a GTP γ S-activated preparation of G_s, which is an equimolar mixture of α -GTP γ S plus the same amount of free $\beta\gamma$, failed to elicit any K⁺ channel openings. Because the $\beta\gamma$ complexes in G_s and those in our PTX-sensitive G protein are the same^{7,8} we emphasize that it is the PTX-sensitive α -GTP γ S complex of G_k, and not the $\beta\gamma$ complex that is responsible for mediating the effect of G_k, and hence for the action of muscarinic receptors on K⁺ channels.

The data of Logothetis *et al.* raise the question as to whether high concentrations of $\beta\gamma$ can indeed activate K⁺ channels in embryonic chick atrial membranes. If so, it will be necessary to determine whether this is related to the ontogenic state of the cardiac muscarinic response, to the fact that avian instead of mammalian cells were used, or to the need for *in vitro* culture of the cells before they are used.

LUTZ BIRNBAUMER
ARTHUR M. BROWN

Baylor College of Medicine,
Houston,
Texas 77030, USA

- Logothetis, D.E., Kurachi, Y., Galper, J., Neer, E.J. & Clapham, D.E. *Nature* **325**, 321-326 (1987).
- Bourne, H.R. *Nature* **325**, 296-297 (1987).
- Yatani, A., Codina, J., Brown, A.M. & Birnbaumer, L. *Science* **235**, 207-211 (1987).
- Hescheler, J., Rosenthal, W., Trautwein, W. & Schultz, G. *Nature* **325**, 445-447 (1987).
- Neer, E.J., Lok, J.M. & Wolf, L.G. *J. biol. Chem.* **259**, 14222-14229 (1984).

6. Rodbell, M. J. *biol. Chem.* **241**, 130-139 (1966).
7. Codina, J., Hildebrandt, J.D., Birnbaumer, L. & Sekura, R.D. *J. biol. Chem.* **259**, 11408-11418 (1984).
8. Hildebrandt, J.D. *et al. J. biol. Chem.* **260**, 14867-14872 (1985).

LOGOTHETIS ET AL. REPLY — Although Birnbaumer and Brown make no such claim in their paper¹, they now maintain that α_{41} is the subunit of G_k which activates the K^+ channel. They reported no experiments with resolved G_k subunits, only with $\alpha\beta\gamma$ -heterotrimers. In contrast, we studied purified α or $\beta\gamma$ and found that the $\beta\gamma$ activates the K^+ channel. We were surprised that $\beta\gamma$ mediated the K^+ channel activation because we also began with the assumption that α would be the only effector. Birnbaumer and Brown view our findings and theirs as necessarily mutually exclusive. We disagree and suggest that the truth may be more complicated and more interesting. Our negative results cannot rule out some role for α subunits, but our positive findings with $\beta\gamma$ at pM concentrations strongly support a role for $\beta\gamma$ in activating the K^+ channel in the heart. The challenge for the future will be to define precise functions for the subunits.

Contrary to the assertion of Birnbaumer and Brown, our results with $\beta\gamma$ are not due to contaminating α protein. The figure shows that 200 pM $\beta\gamma$, a concentration 100-fold lower than we used previously, results in activation equivalent to 200 pM G_k (Fig. 1, ref. 1). We calculate there is less than 0.5 per cent contamination of α in the $\beta\gamma$ preparation, both from densitometry of a highly overloaded SDS-polyacrylamide gel and by pertussis toxin catalysed [³²P]ADP-ribosylation. If a contaminating α is responsible, it must be strongly activating the K^+ channel at less than 1 pM, a concentration below that at which Yatani *et al.* see effects of G_k on the K^+ channel. It is important to stress that any residual α subunit in our $\beta\gamma$ preparations would be unactivated. Our $\beta\gamma$ preparations have never been in GTP γ S. Fluoride activation of the α_{39} protein is readily reversible by removal of fluoride³. Fluoride was removed from the $\beta\gamma$ preparations by one (or two) steps of gel filtration or chromatography before use. Although our results are now shown to occur in the same concentration range as those cited by Yatani *et al.*, there may well be differences due to different patch channel density, detergent, methods of protein determination or sources of atrial membranes and G proteins.

Birnbaumer and Brown suggest that our inability to see activation of the K^+ channel by pure α subunits is because they are denatured or unactivated. We tested the function of the particular preparations of α_{39} and α_{41} used. The α_{39} subunit was activated at 5mM Mg^{2+} , not at 2mM Mg^{2+} . It has a brisk GTPase activity at 22°C which is inhibited by 200 nM GTP γ S. Thus, nucleotides are turning

over at the active site. Although the α subunits were in excess of GTP γ S during activation, we calculate from the K_D (30 nM) that we determined⁴ for α_{39} and α_{41} that the solution applied to the patch contained approximately 9 nM GTP γ S-liganded α subunit, a concentration about 40 times greater than necessary to activate K^+ channels according to Yatani *et al.* The minor (40K) pertussis toxin substrate from brain, described by us⁵ has not yet been purified but is always present in small amounts in the α_{39} preparation.

A strong control for the specificity of the action of $\beta\gamma$ is inhibition of its effect by α subunits, presumably by the formation of inactive heterotrimers. Birnbaumer and Brown speculate that a contaminating protease in the α preparation is responsible. But when we incubate our α with $\beta\gamma$ for 40 min at 30 °C we find no breakdown of either subunit on subsequent SDS-PAGE. Birnbaumer and Brown's suggestion that a protease in the α preparation inactivates a phospholipase in the $\beta\gamma$ preparation is sheer speculation. Finally, the observation that α_{41} decreases Ca channel conductance in NG108 cells⁶ has nothing to do with K^+ channels in heart cells.

Thus we have activated the K^+ channel with picomolar concentrations of $\beta\gamma$ subunits alone. GTP γ S-liganded α subunits at nM concentrations did not activate the channel. Birnbaumer and Brown should test resolved subunits to determine individual activities of their α_{40} and $\beta\gamma$.

DIOMEDES E. LOGOTHETIS

YOSHIHISA KURACHI

JONAS GALPER

EVA J. NEER

DAVID E. CLAPHAM

Cardiovascular Division,
Brigham & Women's Hospital,
Boston, Massachusetts 02115, USA

1. Yatani, A., Codina, J., Brown, A.M. & Birnbaumer, L. *Science* **235**, 207-211 (1987).
2. Logothetis, D.E., Kurachi, Y., Galper, J., Neer, E.J. & Clapham, D.E. *Nature* **325**, 321-326 (1987).
3. Higashijima, T. *et al. J. biol. Chem.* **262**, 752-756 (1987).
4. Huff, R.M. & Neer, E.J. *J. biol. Chem.* **261**, 1105-1110 (1986).
5. Neer, E.J., Lok, J.M. & Wolf, L.G. *J. biol. Chem.* **259**, 14222-14229 (1984).
6. Hescheler, J., Rosenthal, W., Trautwein, W. & Schultz, G. *Nature* **325**, 445-447 (1987).

Climate and reproduction of yellowstone grizzlies

SIR—For more than 50 years before 1968, grizzly bears were permitted free access to several large open-pit garbage dumps inside Yellowstone National Park. Throughout the summer, grizzlies congregated at these sites in large numbers (up to 170 individuals) to feed undisturbed on refuse. Between 1968 and 1971 these dumps were closed abruptly and since then most Yellowstone grizzlies have had to find sufficient natural summer forage to compensate for the loss of garbage. This situation may suit many conserva-

tionists and land managers; but it has almost certainly imposed additional hardship on a population beset with low reproductive rate, high human-induced mortality¹ and severe habitat encroachment.

Picton analysed climate and reproduction of grizzly bears in Yellowstone National Park² and concluded that climatic effects, specifically temperature and precipitation from October to May, were responsible for the decline in grizzly bear litter sizes observed since 1972. We contend that the abrupt closure of the open-pit garbage dumps was a more likely cause of this decline.

To evaluate the relative effects of climate and dump closures on grizzly reproduction properly, climatic factors should be controlled for by analysis of covariance. Using such an analysis, Knight and Eberhardt¹ recently showed that after adjusting for differences in climate, mean annual litter sizes were significantly larger from 1959 to 1970 than from 1971 to 1981. This suggests that the dump closures, rather than climate, probably caused the decline. Our own analysis of covariance shows that when individual litter sizes are adjusted to a common climate index, the mean for the years before the dump closures (1959–69, x_{adj} = 2.17 cubs per litter) was significantly larger than the mean for the years 1973–1981 (x_{adj} = 1.95, P = 0.02; manuscript in preparation). We omitted litters born between 1970 and 1972 because they were conceived during the period of major dump closures (1969–71)³.

Picton² further considered the combined effects of climate and carrion on mean grizzly litter sizes. Because these two predictor variables are undoubtedly inter-correlated, we question the validity of combining the two as he did. It would be more appropriate to control for the effects of each variable statistically, using either path analysis or a partial correlation analysis. We have been unable to do this, however, because neither the carrion data nor the method of deriving an index of relative availability is accessible. The indices themselves are in press⁴, but they include only the values of +1 and -1, suggesting little variation among years in carrion availability. Presumably there are valid data underlying these indices, but Picton has yet to make them available or describe precisely how the indices were derived.

JOHN J. CRAIGHEAD

ROLAND L. REDMOND

Wildlife-Wildlands Institute,
5200 Upper Miller Creek Road,
Missoula, Montana 59803, USA

1. Knight, R.R. & Eberhardt, L.L. *Ecology* **66**, 323–334 (1985).
2. Picton, H.D. *Nature* **274**, 888–889 (1978).
3. U.S. Dept. Interior, Natl. Park Serv., *Proposed Grizzly Bear Management Program, Yellowstone National Park* (1974).
4. Picton, H.D. & Knight, R.R. *Proc. 6th Int. Conf. Bear Res. & Mgmt.* (in the press).

When the Earth moved

Kurt Lambeck

The Ocean of Truth: A Personal History of Global Tectonics. By H.W. Menard.
Princeton University Press: 1986. Pp.353. \$29.50, £19.70.

It is said that in the twentieth century the Earth sciences experienced a revolution; that a series of bold steps provided geology with a physical and philosophical framework into which many previously disparate observations and questions could be slotted. But when did this revolution occur? Was it in 1912, when Wegener put forward his ideas about continental drift? Or did it occur a half-century later with the formulation of the global plate-tectonics model? Despite strong advocates such as DuToit and Holmes, the early ideas on continental drift had little influence on scientific opinion and by the late 1930s the controversy had ended more by exhaustion of the arguments than with firm conclusions — hardly the hallmark of revolution. The formulation of the plate-tectonics hypothesis in the latter half of the 1960s led, however, to an almost overnight conversion to a new set of beliefs about the mobility of the Earth's surface. Staunch anti-drifters became equally strong supporters of the new theory. This, surely, was the revolution.

In writing *The Ocean of Truth*, H. W. Menard — who sadly died not long after completing the book — set out to trace the history of the developments that led from continental drift to plate tectonics, and to summarize the general principles underlying progress in the Earth sciences. Many histories of this time of changing geological thought have been produced by scientists who were not directly involved, and from them one is often left with the belief that progress resulted from flashes of insight from a few brilliant people. The evolutionary nature of the progress is lost sight of and the owners of the toes upon which those remembered stood are forgotten. Menard, in contrast, was ideally placed at San Diego to observe and contribute to the developments in marine geology that were central to the definition of the plate-tectonics hypothesis.

The period after the Second World War was one in which geologists moved from the continents to the deep oceans, the work of the new marine groups at Scripps, Columbia and Cambridge leading to the rapid spread of ship tracks across previously uncharted seafloor. Menard participated in these programmes and made many contributions to the understanding of the morphology of the seafloor and of submarine volcanoes, ocean ridges and fracture zones. He knew

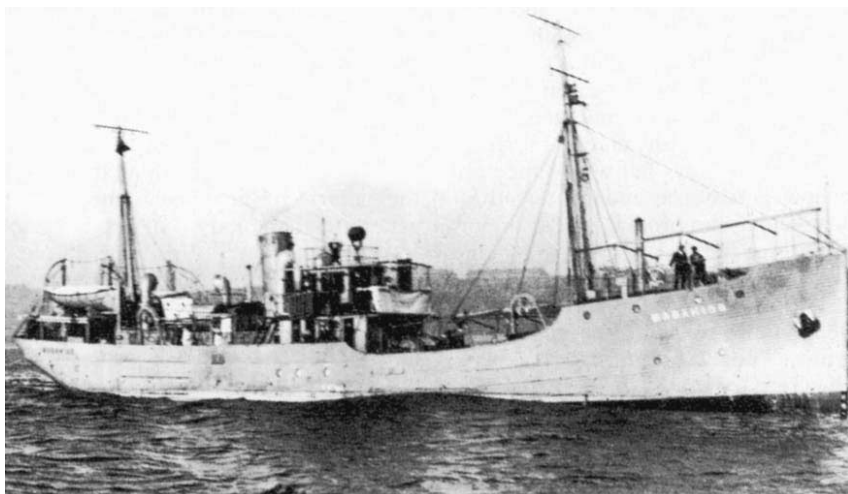
and corresponded with most of the players throughout this period. Above all, he had a memory for detail and a passion for the history of science.

The growth in marine geology in the 1940s and 1950s led to increasingly sophisticated tools to probe the seafloor: improved echosounders, gravimeters and magnetometers, piston corers, explosion seismometers and dredges. It was a time of keeping one's ships at sea and of the collection of much new data. Each new expedition came up with discoveries. It was found that the ocean crust was younger and distinctly different from its continental counterpart; that the sediment cover was generally thin; and that the basement rock was consistently basaltic. Ocean ridges and trenches were charted in detail, and the main fracture zones were discovered and mapped. Most important was the detection of the magnetic patterns of the seafloor. With each passing year, some fragment of geological dogma had to be discarded.

Menard discusses these discoveries almost in passing. His emphasis is upon the scientists involved, and on the rivalry and cooperation between and within institutions, and he looks at the responses of

some of the leading geologists and geophysicists of the time to the early models of seafloor spreading. Which one of the founders of the plate-tectonics model still did not see the significance of the hypothesis of seafloor spreading as late as the end of 1966? As well as giving possible cause for embarrassment to those concerned, Menard traces the development of concepts and interpretations as new surveys were completed. The remarkable magnetic anomaly patterns of the seafloor, for example, had already been recognized by the mid-1950s and the reversals in the magnetic field had been detected. Mason, one of the discoverers of the anomalies and one of the first to attempt to correlate the reversals from core to core, apparently received little support for his ideas from his colleagues. It was to be nearly a decade later, when the groups in Canberra and Menlo Park began to publish their geomagnetic reversal time-scales, that first Morely, and then Vine and Matthews, independently proposed that the magnetic anomalies were clear evidence of seafloor spreading. This was despite the fact that the ideas and papers of Hess and Dietz on seafloor spreading had been in circulation for some years. Cautious acceptance of the seafloor-spreading model then crept into the marine geology community, but its global nature was not yet acknowledged. Seafloor spreading without subduction implies expansion of the Earth, and Menard also discusses the debates that centred on this topic.

The fracture zones were discovered and



Voyage of discovery — the Egyptian ship Mabahiss, the research vessel of the John Murray Expedition of the 1930s, which undertook a major survey of the Indian Ocean and surrounding seas. The picture appears in Deep-Sea Challenge: The John Murray/Mabahiss expedition to the Indian Ocean, 1933–34, edited by A.L. Rice and recently published by UNESCO (British distributor is HMSO). The book is published to commemorate the fiftieth anniversary of the voyages and most of the text is taken up by a narrative by the leader of the expedition Lt-Col. R.B. Seymour Sewell. The upsurge of interest in the Mabahiss because of the anniversary has fostered hopes that the ship may be converted into a floating oceanographic museum of Egyptian marine science.

mapped by Menard himself, and by the late 1950s it was known that there were magnetic anomaly offsets across them. Yet the significance of these great seafloor escarpments as transform faults was only recognized nearly ten years later, in 1965, simultaneously and apparently independently by Coode and Wilson. One contributory development came from seismology: the observation that the earthquakes on transform faults occur only on the section between offset ridges. Indeed, one reason why the key concepts in plate tectonics were formulated when they were was the advances in other areas of the Earth sciences. Progress in isotope geochemistry, experimental petrology and seismology occurred in parallel but without much reference to the events in marine geology; yet without them the plate-tectonics revolution could not have occurred.

Seismology was to play another important role through the work of McKenzie and Parker, who in 1967 published a paper in *Nature* in which the basic elements of plate tectonics were first set forth. Whether or not this was independent of Morgan's work, which appeared a few months later in a journal that takes longer than *Nature* to turn manuscript into print, is debated at length by Menard. He concludes that this is another example of simultaneous discovery, but here the honours were evenly divided. Neither McKenzie and Parker nor Morgan could be as safely ignored as Morely or Coode had been.

In his final chapter, Menard considers the lessons of the history he has described. He notes that those who took to the seas did not play a major role in developing the concepts of seafloor spreading and plate tectonics — this was left to physicists, whose minds were uncluttered by geological detail. Menard also examines why ideas often occurred simultaneously to different scientists; why credit and recognition do not always fall where they are due; how persistence and concerted efforts at selling the product are as important as having the spark of originality; and how publishing quickly and in the right place — and chance with the reviewers — has led to today's common conceptions of priorities. Young scientists can learn much here.

Menard had intended to continue the story up to the present. His death at the age of 66 prevented him from doing so, but it does not distract. Aftermaths of revolution are messy affairs, often dominated by lesser characters. To end with the events leading up to the start of the revolution was appropriate indeed. □

Kurt Lambeck is Professor of Geophysics and Director of the Research School of Earth Sciences, The Australian National University, PO Box 4, Canberra, ACT 2600, Australia.

Technical triumphs

Thomas A. Baillie

Mass Spectrometry in Biomedical Research. Edited by Simon J. Gaskell. Wiley: 1986. Pp. 492. £38.50, \$72.95.

In 1913, J. J. Thomson, the acknowledged father of mass spectrometry, predicted in his treatise *Rays of Positive Electricity and Their Application to Chemical Analyses* that "... there are many problems in Chemistry which could be solved with far greater ease by this than by any other method". History has proved Thomson to be correct. But even he could scarcely have envisaged the enormous impact that mass spectrometry, then in its embryonic stage, was to make on biomedical research in the late twentieth century.

Traditionally, biomedical research has been dependent upon analytical methods with which to elucidate the molecular structure of biologically active substances. It was appreciated early on that the sensitivity and high specificity of mass spectrometry make it especially well-suited to the investigation of structural problems, and to the quantitative determination of trace components of complex biological matrices. But it was not until the late 1960s, when gas chromatography was combined with mass spectrometry and on-line data processing systems were first introduced commercially, that mass spectrometry began to be truly indispensable to the biomedical scientist.

Yet a fundamental limitation remained through the 1970s — the analyte of interest had to be sufficiently volatile (that is, non-polar) to be introduced into the gas phase within the mass spectrometer and undergo ionization. This requirement effectively restricted the accessible mass range of analytes to around 1,000 daltons, a severe limitation indeed.

This picture changed dramatically with the advent of new techniques for the generation and analysis of gas-phase ions from samples in the condensed phase. These powerful new methods permit the study of biological molecules which are neither volatile nor thermally stable. As Dr Gaskell states in his preface to the book under review, "The impact on biomedical research has been such that the advancement of mass spectrometric techniques and of certain areas of biomedical research are now inextricably linked and the demands of the biochemist constitute the principal driving force in the development of mass spectrometry". The purpose of this volume, therefore, is to impart a sense of the current state of play in the field, and a glimpse of future directions.

The book is divided, somewhat arbitrarily, into three sections, each of which

is preceded by an introduction from the editor. Part I covers the analysis of labile and polar compounds of biological interest, and includes discussions of the role of mass spectrometry in studies of leukotrienes, bile salts, nucleic acid constituents, acyl carnitines, coenzyme A thioesters and polar drug metabolites.

Part II, the heart of the volume, focuses on analyses at high mass, and deals largely with the relative merits of different ionization techniques and mass analysers for the investigation of molecules in the range beyond 1,000 daltons. Examples are drawn from work on various biological oligomers, including peptides, carbohydrates, glycoconjugates and nucleic acids, and practical aspects of the analysis of these complex biopolymers are discussed by several of the contributors. The book concludes with a section on trace analyses, where mass spectrometry has been employed for the quantitative determination of relatively low-molecular-weight analytes, of both endogenous and exogenous origin, in biological fluids and tissues at the parts-per-billion level and below.

Despite the inevitable problems with continuity in a book of this type, Dr Gaskell has done an admirable editorial job. The 26 chapters are written by active researchers and are well-balanced, clearly presented and appropriately illustrated. Together they give an accurate impression of the current capabilities (and limitations) of mass spectrometry for research in the biomedical sciences. The technique has indeed come a long way since the observation of "rays of positive electricity" derived from helium and other inert gases. Protein biochemistry has arguably been the primary beneficiary of these advances; recent accomplishments have included the analysis of enzymes such as porcine trypsin, which was determined by ²⁵²Cf-plasma desorption time-of-flight mass spectrometry to have a molecular weight of 23,406 ± 140 daltons. Were J. J. Thomson still alive, he would surely be impressed. □

Thomas A. Baillie is an Associate Professor in the Department of Medicinal Chemistry, University of Washington, Seattle, Washington 98195, USA.

New in paperback

- *The Intelligence Men: Makers of the IQ Controversy* by Raymond E. Fancher. Publisher is W.W. Norton, price is £4.95, \$7.95. For review see *Nature* **318**, 113 (1985).
- *Concise Science Dictionary* edited by Alan Isaacs, John Daintith and Elizabeth Martin. Publisher is Oxford University Press, price is £5.95, \$8.95 (to be published in July in the United States). For review see *Nature* **312**, 670 (1984).
- *The Oxford Companion to Animal Behaviour* edited by David McFarland. Publisher is Oxford University Press, price is £12.95 (\$19.95; to be published in July). For review see *Nature* **294**, 501 (1981).

Current research

James L. Gould

Electroreception. Edited by Theodore Holmes Bullock and Walter Heiligenberg. Wiley: 1986. Pp.772. £95.45, \$99.95.

MOST multi-author volumes are, let's face it, a pig's breakfast of papers of wildly varying length, style, scientific quality and relevance to the overall theme concerned; they represent the usual compromises between whom the organizers ought to have invited to contribute, those they did invite, those that actually agreed to prepare something, and the second stringers invited at the last minute to fill in the gaps. To make matters worse, the editing of such papers is ordinarily the purest embodiment of *laissez-faire* known to publishing, while the cost per page of such volumes is breathtaking. It is a delight, therefore, to encounter a notable exception.

Electroreception is, for the most part, a well-organized, systematic explication of what is known about electroreception in fish. It begins with the central generation of output (the circuits that generate electric-organ discharges), follows this information to the periphery (the electric organs themselves), then picks up the story with the receptors, follows the information back in to the brain where it is analysed, and then returns to the periphery where behavioural responses are effected. The main anomaly in this linear order of topics is a contribution on spinal cord regeneration, which bears no relation to the other 20 papers.

Beyond the logical order of what will surely become the standard reference work on electric fish, the book has a thoroughness and depth of detail that set it apart from others of its species. For example, the discussion of electroreceptors is divided up into eight chapters, each treating a specific group of fish. (There is also a chapter on electroreception in amphibia, though none on the recent discovery of an electric sense in the platypus, a report which seems to be dismissed in the introduction as lying somewhere between pure imagination and plausible speculation.) Given that electroreception appears to have evolved independently several times among fish, this is the only sensible approach. The editors have obviously insisted on a high standard of writing and anatomical illustration.

In looking for something to criticize, I find my only real complaint is that less than full justice is done to behaviour. The chapters by Hopkins and Hagedorn which relate the ecology and behaviour of electric fish to their special sensory world are delights, and Heiligenberg's masterly description of the neural basis of jamming-

avoidance behaviour is the high point of the volume for me. But where is Kalmijn's work on magnetic-field orientation by means of electroreceptors, to mention the most obvious omission? Alas, the relatively small portion of the book devoted to behaviour to some degree mirrors the emphases of the field.

The greatest progress in neuroethology has been made by examining specialist species, organisms such as bats and owls whose behaviour, sensory structures and neural wiring have evolved to tackle one principal task. The first step is always behavioural, devising experiments that define what the animal is doing to solve the problems unique to its lifestyle. These observations tell us what the nervous system must be doing, and suggest how it does it. Informed by the behavioural work, efficient investigation of the neural

bases of behaviour becomes possible. (It has always seemed unfortunate that so much neurobiological work has concentrated upon generalist species such as cats and monkeys, and so has lacked this evolutionary guidance to hypotheses and interpretation.) As this volume demonstrates, there has been more emphasis on the special sensory structures than on the behaviours they serve; at the same time, the magnificent story of the jamming-avoidance response, which is traced from receptor to CNS to effector in more detail than perhaps any other behavioural pathway in neuroethology, demonstrates the power and potential of specialist species in making possible a deep understanding of the neural bases of behaviour.

James L. Gould is a Professor in the Department of Biology, Princeton University, Princeton, New Jersey 08544, USA.

Tumour history

A. Rosalie David

Palaeo-oncology: The Antiquity of Cancer. Edited by Spyros Retsas. Farrand Press, 50 Ferry Street, Isle of Dogs, London E14 9DT, UK:1986. Pp.58. £9.50.

THE IDEA of an international meeting about the antiquity of cancer, first proposed by Professor G. Daikos of the University of Athens, came to fruition in 1984 when the topic was incorporated in the IV Mediterranean Congress of Chemotherapy. Abstracts of the relevant papers were included in the proceedings of the congress, but participants also wished to see these contributions produced as a separate volume. The resulting book, which has been produced with support from Lederle Laboratories, includes four contributions by the symposium panellists.

In the first paper, G.P. Stathopoulos considers the evidence of tumours found in fossilized remains and early skeletons, including those of Saxon, Peruvian, Danish, Egyptian and African populations. Despite the difficulties of examining some skeletal parts by pathological and roentgenological methods, there are clear indications of the presence of benign and malignant tumours in antiquity. P. Ghalioungui then deals with the subject of malignancy in ancient Egypt. He shows that evidence can be derived from iconography, palaeopathology or literary sources, and quotes some examples.

The third paper, by F. Sweha Boulos, looks at oncology in the Egyptian papyrus, and discusses the use of terms related to tumours and the description of symptoms. Finally, S. Retsas considers the precise definitions of cancer and its malignant nature as manifested in ancient Greek

writings, including those of Hippocrates and Galen.

Several important points emerge from the book. The incidence of tumours in antiquity would appear to have been relatively low, and the variety of them that afflict modern man was missing. This may be because the development of some neoplasms is age-related, and in antiquity few individuals lived beyond middle-age. Also, the study of ancient remains is usually limited to osseous material, although the preserved viscera of Egyptian mummies may be a useful area for future research. The development of new techniques will ensure that specimens held for many years in reserve stores can be re-examined and successfully re-evaluated; thus, the preservation of such material is of great importance.

Questions also arise over the effects of industrial pollution and diet on the incidence of cancer. The disease afflicted man well before pollution became such a serious problem, and in a range of populations which had diverse dietary habits.

In compiling this book, the authors hoped to stimulate interest in palaeo-oncology and to show how ancient literary and palaeopathological sources can illuminate the history of cancer and perhaps assist in understanding the disease today. The papers will undoubtedly encourage medical researchers and palaeopathologists to explore the subject further, and will also provide points of interest for ancient historians. It would, however, have been of help to the general reader if the passages from Hippocrates and Galen (quoted in Greek) had been accompanied by an English translation, and if a brief bibliography had been included to augment the references given at the end of each paper. □

A. Rosalie David is at The Manchester Museum, University of Manchester, Manchester M13 9PL, UK.

Gone to the moons

Lionel Wilson

Satellites. Edited by Joseph A. Burns and Mildred Shapley Matthews. *University of Arizona Press: 1986. Pp. 1,021. \$55.*

LONG GONE are the days when scientists interested in the Solar System took planetary satellites to be insignificant companions of the main objects of interest. In modern planetary science it is firmly acknowledged that there is at least as much to learn about the origin and evolution of the Solar System from the study of the smaller bodies, and in particular the natural satellites, as there is from examination of the planets themselves.

Relative to the planets, the satellites exhibit a slightly wider range of sizes and a much wider range of bulk compositions. Their evolutionary histories have in most cases been influenced not only by their distances from the Sun, but also by the proximity and evolutionary history of their 'parent' planets through such processes as direct heating, tidal heating, tidal deformation and collision with other satellites or trapped cometary bodies. In particular, the availability of heat sources other than those that drive planetary thermal histories (radioactive decay and core formation), has led to evolution of satellite surfaces that might otherwise have been expected to remain in the states in which they originally accreted.

Yet the study of satellites could not go ahead without reliable and suitably high-resolution observational data. As recently as the mid-1970s, only the Earth's Moon could be investigated in any great detail. This state of affairs changed dramatically with the advent first of the Viking 1 and 2 missions to Mars, which sent back images of that planet's two tiny moons, and then (and much more importantly) of the Voyager 1 and 2 missions, which have

provided detailed information on the satellite systems of Jupiter, Saturn and, most recently, Uranus.

The timing of the reception of data from the successive Voyager fly-by events is dictated by the nature of the trajectory used to cross the Solar System, and it is inevitable that there is a gap of a few years between successive encounters. For some, these gaps have been frustrating. But one suspects they have had a positive influence on the data analysis programme that has paralleled the missions. There has been time to assimilate new data, formulate fresh models and define what observational improvements might be made at the next encounter; graduate students associated with one phase of the mission have become junior team members by the next; and there has been a continuity of attention from the theoreticians which has itself been an important factor in the advance of many aspects of the research.

All of these subtleties are in evidence in this excellent set of review and discussion papers, the latest in a long series of such volumes fostered by the University of Arizona. Based on a conference held in 1983, the contents nevertheless run up to late 1985 (and early 1986 for the Uranus encounter). Particularly striking is the range of coverage, from the origins, internal structures and evolutionary histories of the satellites, through their present surface states, to their interactions with one another, their parent planets and the interplanetary environment. This collection will be essential reading for all students of the Solar System. It will also do much to make the wider scientific community aware of the exciting state of planetary research. □

Lionel Wilson is a Senior Lecturer in the Department of Environmental Science, University of Lancaster, Lancaster LA1 4YQ, UK, and a Professor at the Hawaii Institute of Geophysics.

Floristic features

Peter D. Moore

The Savannas: Biogeography and Geobotany. By Monica M. Cole. *Academic: 1986. Pp. 438. \$79.50, £46.*

SAVANNA covers 20 per cent of the Earth's land surface, and provides a resource for vast numbers of domestic animals and wildlife. It is therefore a biome worthy of note. But controversy surrounds the definition, classification, mode of origin and maintenance of these tropical grasslands and parklands. All studies on their value as natural resources hang upon a commonly accepted terminology and use of vocabulary, and it is one of Monica Cole's aims to provide that framework.

She begins with definitions and generalities, and then moves on to detailed accounts of the savanna vegetation of South America, Africa, India, South-East Asia and Australia. The most distinctive feature of these descriptions is the concentration upon floristic features rather than on the ecosystem as a whole. Little is said about primary production, patterns of energy flow or nutrient cycles, but the vegetation is described in impressive geographical and botanical detail. This says much for the wide field experience of the author, and it puts her in an authoritative position to advance the definitions proposed at the beginning of the book.

In addition to the descriptive material, Cole puts forward some strongly held views on the environmental determinants of savanna. Much speculation surrounds this subject, mainly centred on the relative roles of climate, soils and man (including the grazing of stock and the use of fire) in bringing the savannas into being

and maintaining them in their relatively open state. In the past there has been emphasis upon the sharp boundaries that often exist between savanna and forest, leading to the conclusion that fire is the primary causative factor in the maintenance of savanna. Cole, by contrast, stresses the importance of geomorphological and climatic factors. Her attitude can be summarized by her comment about West Africa: "notwithstanding the importance of fire, vegetation distributions including those of forest and savanna and of different categories of savanna are controlled by interacting physical factors upon which fire and man's cultural practices impose a secondary influence".

Savanna occurs in regions with summer rain and winter drought. Where the water supply and moisture retention in the soil is inadequate to support forest, savanna develops. Grazing, according to Cole, may be disregarded as a primary determinant, because savanna is found both in continents with rich herbivorous faunas, such as Africa, and poor ones, such as Australia. Such influences may have affected the evolution of spiny forms of growth, but not the general physiognomy of the savannas.

Cole's position on savanna development is argued throughout the book with conviction and with abundant local illustrations. She presents a strong case, but one detects an element of partisan spirit — perhaps even bias — in her complete neglect of the human factor in her diagrammatic model of savanna-environment relationships. This comes in the opening chapter, and is a disturbing introduction to a book which is far more balanced than the diagram may lead one to expect. □

Peter D. Moore is in the Department of Biology, Kings College London (KQC), Campden Hill Road, London W8 7AH, UK.

New journals review

On 24 September *Nature* will publish the seventh annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1987 issue are that:

- (i) the first number appeared, or the journal was retitled, *between June 1985 and May 1986* (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);
- (ii) it is published at least three times a year;
- (iii) the main language used is English.

Publishers and learned societies are invited to send *four different issues of each suitable periodical, including the first and most recent numbers* (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details for 1987 (and 1988 if possible) should be included.

Rotation and precession of comet Halley

Klaus Wilhelm

Max-Planck-Institut für Aeronomie, D-3411 Katlenburg-Lindau, FRG

Measurements obtained during the recent appearance of comet Halley suggest that its nucleus undergoes free and forced precession motions. Periodicities of 2.2 and 7.4 days can be explained by a 2.2-day rotation about the minor axis and a free-precessional period of 14.8 days.

THE spacecraft encounters with comet Halley in March 1986 have, for the first time, provided detailed information on the size and the shape of the cometary nucleus and its orientation in inertial space¹⁻⁶. This, as well as other space and ground-based observations obtained during the 1985-86 apparition of the comet, has led to many attempts to improve our knowledge of the rotation characteristics of the nucleus. It appears as if the concept of a pure rotation of a rigid body about its axis of maximum moment of inertia is not sufficient to describe all observations. The findings concerning the elongated shape and the concentration of the sublimation process in localized jets^{1,2} make it unlikely that such an undisturbed rotation would be a realistic assumption. Spacecraft data have also provided better estimates of the mean density of the nucleus that must, because of its large size and the constraints on the total mass^{7,8}, be much less^{9,10} than previously assumed. Direct measurements of the gas and dust production rates and the neutral gas expansion velocity¹¹⁻¹⁶ allow estimates of the jet reaction forces and torques to be deduced. These observations indicate that the rotation characteristics are more complicated than was thought on the basis of the 1910 and pre-encounter data¹⁷⁻²⁰. This is not unexpected as studies of other comets, such as Encke, have shown that non-gravitational forces can be related to forced precession of cometary nuclei²¹.

Evidence was presented at the Heidelberg symposium on the "Exploration of Halley's Comet" in October 1986 that suggested a periodicity of $P_0 = 7.4$ days in the coma brightness and was interpreted as the rotational period of the nucleus (ref. 22; M. C. Festou *et al.*, personal communication). This is in conflict with many other observations favouring a rotation period of approximately 2.2 days^{1,3,5,6,17,19,20}. Sekanina²³ recently proposed a model that involved a rotation period of 7.4 days about the long principal axis of the nucleus as well as free precession with large cone angles and a period of ~ 2.2 days. On the basis of the spacecraft observations, a 7.4-day rotational period has subsequently been rejected by Smith *et al.*²⁴ for rotations both about the shortest and longest principal axis.

In this situation, it might be of interest to go one step beyond the consideration of idealized models and attempt a numerical integration of Euler's equations in their general form. The observations available should allow us to arrive at refined models for the attitude characteristics of the nucleus of comet Halley.

Model assumptions

The nucleus will be approximated by a homogeneous triaxial ellipsoid

$$(x/a)^2 + (y/b)^2 + (z/c)^2 = 1 \quad (1)$$

in a body-fixed coordinate system X, Y, Z . The lengths of the principal axes X and Z can be defined relatively accurately from spacecraft measurements as $2a = 1.6 \times 10^4$ m and $2c = 8.2 \times 10^3$ m, respectively¹⁻⁵. The Y -axis has been estimated from Vega 1 images to be $2b \approx 8 \times 10^3$ m (K. Szegő, personal communication). Deviations of the shape of the real nucleus from this model, which may be important in other studies, are of no relevance in this context. The encounter observations demon-

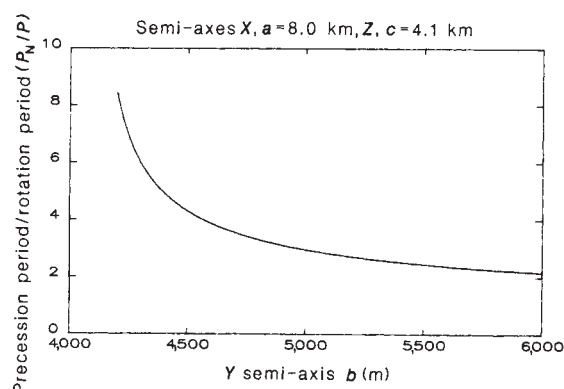


Fig. 1 Dependence of the ratio P_N/P (P_N is the free-precession period for small cone angles and P is the rotation period) on the length of the medium principal semi-axis b of a homogeneous tri-axial ellipsoid with semi-major axis $a = 8,000$ m and semi-minor axis $c = 4,100$ m. A ratio of 3.36 ($P_N = 7.4$ and $P = 2.2$ days) requires $b = 4,770$ m (case 1) and a ratio of 6.72 ($P_N = 2 \times 7.4$ days) leads to $b = 4,260$ m (case 2).

strated, in addition to the elongated shape of the nucleus, that the jet activity was strongly concentrated near the subsolar region²⁵. The most likely direction of the rotation axis near a right ascension of $\alpha = 50^\circ$ and a declination of $\delta = -45^\circ$ (epoch 1950.0 of celestial coordinates) implies an obliquity of $I = 20$ to 30° ^{5,6} resulting in significantly asymmetric jet reactions on the nucleus. Hence, the motion of the nucleus cannot necessarily be considered as torque-free. Note that the rotation axis so determined is approximately pointing in the $-Z$ direction. Because a stable rotation of a rigid body about its medium axis is not possible it follows for the semi-axes

$$a \geq b \geq c \quad (2)$$

The principal moments of inertia

$$A = (b^2 + c^2)\mu/5 \quad B = (a^2 + c^2)\mu/5 \quad C = (a^2 + b^2)\mu/5 \quad (3a-c)$$

where $\mu = 4\pi abc/3$ is the mass of a homogeneous ellipsoid with density ρ , consequently obey the inequality

$$A \leq B \leq C \quad (4)$$

It is worth mentioning at this stage that the components of the inertia tensor of any rigid body will comply with inequality (4), if the orientations of the axes are chosen appropriately, and thus the specific choice of the shape of the nucleus does not entail a loss of generality as far as dynamic studies are concerned.

For small precession angles the period of free precession P_N can be approximated by

$$P_N \approx P/\sqrt{(C/A-1)(C/B-1)} \quad (5)$$

where P is the period of rotation about the Z -axis²⁶. The ratio P_N/P is shown in Fig. 1 as a function of the medium semi-axis.

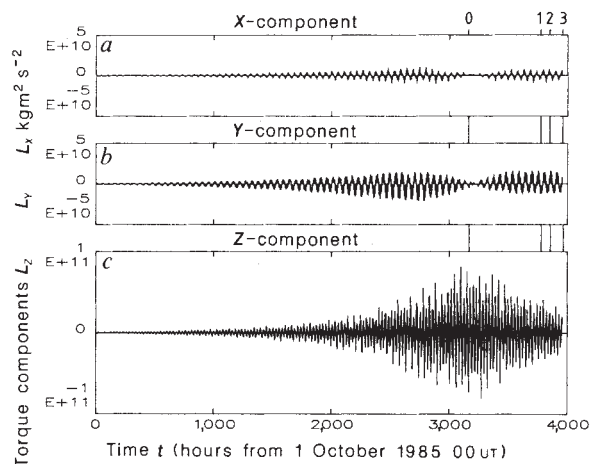


Fig. 2 *a-c*, Torque components in the body-fixed system *X*, *Y*, *Z* respectively as a function of time counted from 1 October 1985, 00 UT. The assumptions that led to the torques shown here ($Q_{\max}/Q_{\min}=1.4$ and randomly fluctuating jets) are described in the text. The perihelion is marked (0) as well as the spacecraft encounters (Vega 1, 1; Vega 2, 2; Giotto, 3).

It can be seen that P_N/P is strongly dependent on the length of b for $b \rightarrow c=4,100$ m. If the 7.4-day periodicity observed could be associated with a precessional motion of the nucleus, as has been suggested at the Heidelberg meeting^{6,20} and by Julian²⁷, this would, together with a rotation period of 2.2 days, determine the length of the medium semi-axis to be $b=4,770$ m (case 1). This value is larger than that observed by Vega 1. Even if the error margin of the absolute length of b , as estimated from Vega 1 images, could accommodate this value, there would still be a discrepancy in the relative measure of b/c . This ratio would be 1.16 for case 1. It was however found to be between 1 and 1.07 (refs 5, 24; K. Szegö, personal communication). A ratio $b/c=1.04$ has, therefore, been chosen here in order to obtain a precessional period of 2×7.4 days for a length of the medium semi-axis of $b=4,260$ m (case 2). The reason for this particular choice will become clear in the discussion section.

Euler's equations can be written as

$$\begin{aligned} A\dot{p} + (C-B)qr &= L_x, & B\dot{q} + (A-C)rp &= L_y, \\ C\dot{r} + (B-A)pq &= L_z \end{aligned} \quad (6a-c)$$

where p, q, r are the components of the angular velocity vector Ω in *X*, *Y*, *Z* and L_x, L_y, L_z are the corresponding torque components. To integrate equation (6a-c), the initial conditions and the torque components have to be established. Under the assumption that the attitude of the nucleus did not change

drastically during the perihelion passage (a premise that could be checked in the course of this analysis), the orientation of the rotation axis during the spacecraft encounters should provide a reasonable estimate of this parameter. It will further be assumed that any free precession of the nucleus excited during the previous apparition was damped during the 1.26×10^4 rotations with $P=2.2$ days which occurred within one orbital revolution. The nucleus was thus stably spinning about its *Z*-axis on 1 October 1985, the somewhat arbitrarily selected starting point of this study. The heliocentric distance of the comet was $R=2.34$ AU at that time.

The determination of the torque components is much more involved. The sum of the mean gas and dust production rates of comet Halley at the time of the Giotto encounter was approximately $Q_G=2 \times 10^4 \text{ kg s}^{-1}$,^{13,28} while this rate was a factor of 1.5 to 2 higher for Vega 1 and Vega 2²⁹. Opposite ends of the long axis pointed towards the sun during the Vega 1 and the Giotto encounters^{5-7,24}. The large changes of the production rates observed will be interpreted to mean that one side of the nucleus was more productive than the other, in agreement with earlier conclusions reached by Sekanina and Larson³⁰. Here a ratio of $Q_{\max}/Q_{\min}=1.4$ will be used for the opposite ends of the long axis. The mean production rate Q was assumed to vary in accordance with the non-gravitational forces and hence was proportional to

$$g(R) = \alpha(R/R_0)^{-8}[1 + (R/R_0)^n]^{-k} \quad (7)$$

as given by Marsden *et al.*³¹ with $\alpha=0.1113$, $R_0=2.808$ AU, $g=2.15$, $n=5.093$ and $k=4.6142$. It was normalized to $Q_0=1.54 \times 10^4 \text{ kg s}^{-1}$ at $R=1$ AU.

At any instant, the actual production rate probably fluctuated about a mean value as a consequence of irregular surface features and inhomogeneities of the nucleus. The calculations have, therefore, been performed with varying production rates randomly and equally distributed with mean values of $0.83Q$ and $1.17Q$, respectively. The gas expansion velocity was measured to be $v_E=900 \text{ m s}^{-1}$ ¹³. Only a certain fraction ϵ_1 of the expansion velocity contributed to the reaction forces, while the remaining portion $1-\epsilon_1$ can be attributed to the free expansion of the gas in the inner coma. Reactions of the jets on the nucleus resulted in torques, when the jet axes were not passing through its centre of gravity. The asymmetric portion ϵ_2 of the sublimation, in all likelihood concentrated near the subsolar point, is thus of interest in this study. Combining ϵ_1 and ϵ_2 in an efficiency number $\epsilon = \epsilon_1 \epsilon_2$, the forces effective for torque generation can be written as

$$F_{\text{eff}} = \epsilon v_E Q \quad (8)$$

A value of $\epsilon=0.2$ was used here, but it is realized that this choice can only be considered as a guess. Finally, the torque

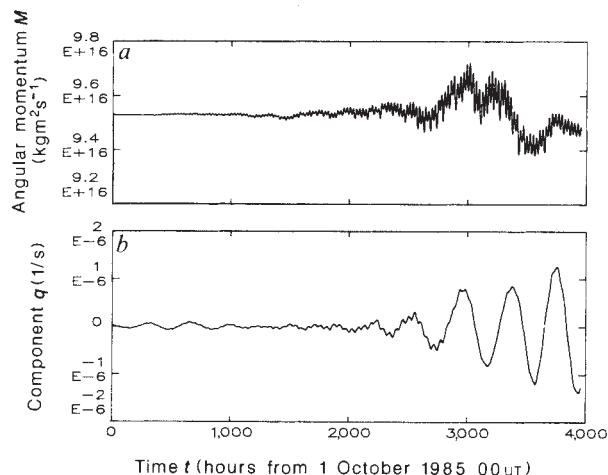
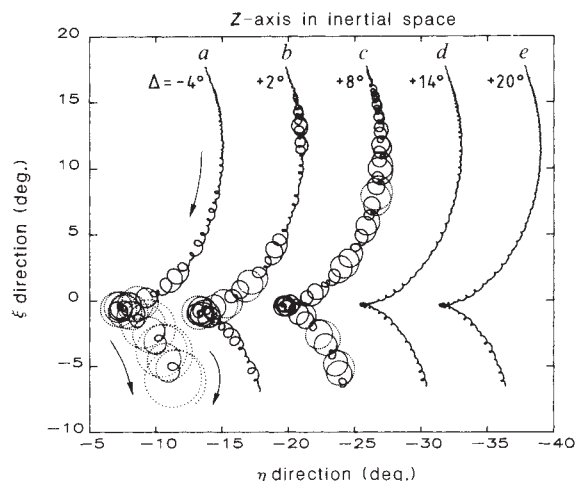


Fig. 3 *a*, Total angular momentum variations and *b*, component q of the angular velocity vector as functions of time obtained in response to the application of the torques shown in Fig. 2*a-c* with no damping.

Fig. 4 The motion of the Z-axis of the model nucleus in inertial space (coordinate system ξ, η, ζ) under five conditions. The curves have been obtained as follows: *a*, random and asymmetric jet activity as described in main text without damping; *b*, random and asymmetric with damping time constant of 100 days; *c*, random and symmetric without damping; *d*, asymmetric without damping; *e*, symmetric without damping. The directions of motions caused by forced and free precession are indicated by arrows. The diagram was produced by calculating the Eulerian angles θ (angle between ζ and Z) and ψ (angle between ξ and line of nodes) of the Z-axis every 190 s and plotting every 20th point as angular deviation from the ζ axis in the ξ - η plane. The curves have to be displaced in the η direction by the values of Δ given in the figure to get their correct positions.



arms of the asymmetric jet reactions have to be obtained. As mentioned above, it will be assumed that the jet activity was near the subsolar point. A configuration has been chosen that facilitated the numerical calculations by specifying that the force be effective at the point in the X - Y plane that was closest to the sun. In order to study the influence of the model assumptions, runs were also performed without $Q_{\max}/Q_{\min}$ asymmetry and/or randomly fluctuating jets.

Integration of Euler's equations

Equation (6a-c) can now be integrated for certain combinations of parameter sets. Based on the arguments given above, results obtained for $b = 4,260$ m, $P = 2.2$ days (sidereal), $I = 25^\circ$, $\rho = 300 \text{ kg m}^{-3}$ and $\varepsilon = 0.2$ will be considered in particular. The iteration time will be $P/1,000 = 190$ s. The volume of this body would be $V = 5.85 \times 10^{11} \text{ m}^3$ with a mass of $\mu = 1.76 \times 10^{14} \text{ kg}$ and principal moments of inertia of $A = 1.23$, $B = 2.84$ and $C = 2.88 \times 10^{21} \text{ kg m}^2$. Its angular momentum amounts to $M_0 = 9.54 \times 10^{16} \text{ kg m}^2 \text{ s}^{-1}$ and the rotational energy is $T_0 = 1.58 \times 10^{12} \text{ J}$.

Figure 2a-c shows the torque components L_x , L_y , L_z about the principal axes as a function of time from 1 October 1985, 00 UT up to spacecraft encounters in March 1986. The spin modulation can be seen in L_x and L_y , superimposed by random jet activity. The minima near $t = 3,200$ h in X and Y result from the fact that the Sun was crossing the X - Y plane at that time for the selected initial conditions of the rotation axis direction. The general development of the magnitude of the jet reactions during the perihelion passage is evident from Fig. 2c showing the Z component of the torques. In Fig. 3a, the total angular

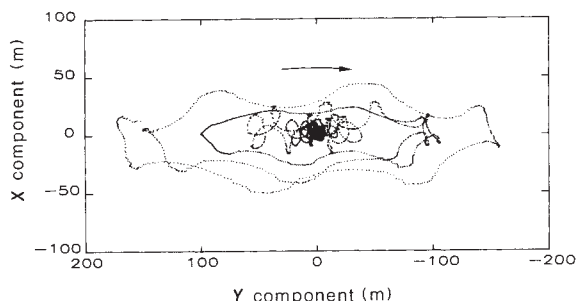


Fig. 5 Terminus of the angular velocity vector on the surface of the model nucleus (polhodes). As in Fig. 4, points have been plotted every 3,800 s. The Z -axis is in the centre of the diagram. Both the free precession with a period of 14.8 days (direction indicated by arrow) and the forced oscillations can be distinguished (compare also Fig. 3b).

momentum is presented as a function of time to demonstrate that variations of about 3% have to be expected for this parameter. Figure 3b shows, as an example, the q component of the angular velocity vector. Both forced oscillations with a synodic period of $P_s \approx P$ and free precession with period P_N and increasing amplitudes can be recognized in the diagram.

The motion of the nucleus under the influence of jet reactions has been plotted in Fig. 4 for five model assumptions. The position of the Z -axis is shown in inertial space represented by a rectangular coordinate system ξ, η , where ξ and η lie in the orbital plane of the comet with ξ pointing away from the Sun at perihelion. Forced precession motions are present in all cases. The conspicuous reversals occur, when the Sun passes through the X - Y plane (see Fig. 2a, b). The forced precession induced angular variations of about 30° . This motion is neither dependent on the $Q_{\max}/Q_{\min}$ asymmetry nor on the random activation of the jets. On the other hand, free precession with half-cone angles of up to 2° is only initiated when the jet action is randomly occurring (curves a-c). The $Q_{\max}/Q_{\min}$ asymmetry has no major impact as can be seen by comparing curves a and c. A damping time constant of 100 days applied after each iteration does not change the situation significantly before perihelion, but gives much smaller cone angles thereafter (curve b). Equations (3a-c) and (6a-c) imply that the cone angles of the precessional motion are inversely proportional to the density ρ for given torque components. Because of the random nature of the jet reactions, different runs with the same model assumptions and initial conditions do not give exactly the same results. The paths shown in Fig. 4 are however representative examples. It might also be of interest to demonstrate the motion of the angular velocity vector on the surface of the model nucleus, which is done in Fig. 5 for case a. As can be seen, this vector moves up to 180 m away from the Z -axis. The deviations are larger in $\pm Y$ directions than in $\pm X$ directions, which is a consequence of equations (6a, b) and the fact that $C \approx B > A$. Equation (6a) with $L_x = 0$ also shows that a precessional motion of Ω about a small axis is not possible for a symmetric top with $C = B$.

Discussion

If Smith *et al.*²⁴ are correct in rejecting a 7.4-day rotation period hypothesis both for the long and the short axis, other ways of explaining the observations have to be studied. One possibility that has been mentioned is a 'moderately deformable nucleus'^{9,27}. Although the present study is based on the assumption of a rigid body, it appears that small deviations from this concept should not invalidate the findings on the dynamical characteristics of the nucleus. It was already postulated earlier in this paper that internal dissipation should have damped any free-precessional motion excited during the previous apparition.

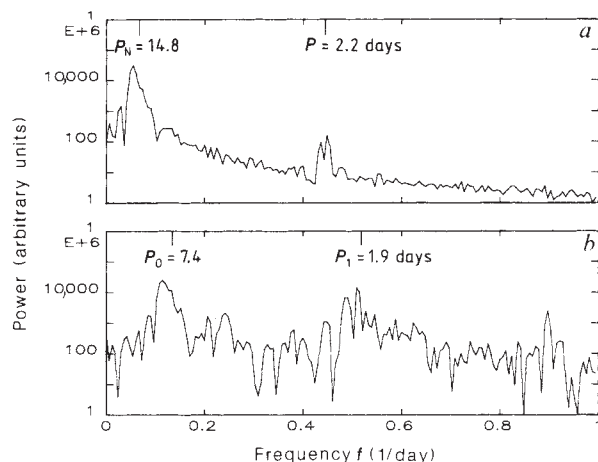


Fig. 6 Power spectra of *a*, the component *q* of the angular velocity vector and *b*, the speed of the terminus of Ω on the surface of the nucleus expressed by $v/c \approx d(\sqrt{p^2 + q^2}/r)/dt$. The relevant peaks are also labelled with the corresponding periods for easy reference.

A maximum time constant compatible with achieving this damping would be of the order of 20 years. If, on the other hand, the damping time would be equal or greater than about 100 days, then the influence on the motion of the nucleus during the perihelion pass would be small as has been shown in Fig. 4. There is consequently a wide range of damping times that would fulfil both requirements.

A deformable and dissipative body would have to adjust itself to stresses caused by movements of the angular velocity vector in the body-fixed system *X*, *Y*, *Z*. Maximum stresses caused by centrifugal forces amount to only 10 N m^{-2} . The stress variations will to some extent control the gas production rate if the body is very fragile. Highest production rates would be expected, when the terminus of the angular velocity vector moves fastest, which of course occurs twice per precession period. The power spectrum of the speed of the terminus of the angular velocity vector, shown in Fig. 6*b*, has indeed a strong peak at $P_N/2 = 7.4$ days, in addition to that near $P_1 = 1/(1/2.2 + 1/14.8)$ days. The coma brightness variations reported by Millis and Schleicher²², if interpreted in the context of this model, would thus be understandable with the choice of $P_N = 14.8$ days made in case 2. The power spectrum of the component *q* is given in Fig. 6*a* for comparison. It is dominated by the rotation and precession periods P_S and P_N .

The partial control of the gas production rate by variations of Ω could also contribute to the observed post-perihelion enhancement of the light curve of comet Halley³²⁻³⁴. Figures 3*b* and 4 (curve *a*) show marked differences of the attitude characteristics of the nucleus before and after perihelion. With relatively weak internal dissipation, directional variations of Ω would continue for some time and could maintain a higher

outgassing rate than would be expected from thermal considerations.

Conclusions

In an attempt to model the attitude characteristics of the nucleus of comet Halley, it was found that forced precession will change the direction of the *Z*-axis of the body system in inertial space by approximately 30° during the perihelion passage up to the spacecraft encounter times. An initialization of free precession with half-cone angles of more than 2° is not very likely, unless the mean density is much lower than assumed here. Any suggestion to explain the observed periodicity of 7.4 days as an effect of solar-aspect variations oscillating with the free-precession period would place very specific requirements on the surface of the nucleus. A small variation of the *Z*-axis orientation in space would have to bring very active centres in and out of the Sun-illuminated area. Even if this could happen occasionally, the variable solar-aspect angle changing by forced precession would prevent this becoming a recurrent feature. With the assumptions made such a suggestion therefore cannot provide an explanation. A non-rigid nucleus, on the other hand, whose gas production rate is partially controlled by stress variations during the free-precessional motion, could lead to the 7.4-day periodicity observed as well as to the post-perihelion brightness increase.

Many stimulating discussions with members of the Halley Multicolour Camera and the Vega imaging teams are gratefully acknowledged. The study was financially supported by the German Bundesministerium für Forschung und Technologie, the Max-Planck-Gesellschaft and the Deutsch Forschungs- und Versuchsanstalt für Luft- und Raumfahrt.

Received 26 January; accepted 18 March 1987

1. Sagdeev, R. Z. *et al. Nature* **321**, 262-266 (1986).
2. Keller, H. U. *et al. Nature* **321**, 320-326 (1986).
3. Sagdeev, R. Z. *et al. Adv. Space Res.* **512**, 95-104 (1985).
4. Reitsema, H. J. *et al. Proc. 20th ESLAB Symp.* Vol. II. Heidelberg, ESA SP-250 (eds B. Batrick, E. J. Rolfe & R. Reinhard) 351-354 (European Space Agency, Noordwijk, Netherlands, 1986).
5. Sagdeev, R. Z. *et al. Proc. 20th ESLAB Symp.* Vol. II. Heidelberg, ESA SP-250 (eds B. Batrick, E. J. Rolfe & R. Reinhard) 335-338 (European Space Agency, Noordwijk, Netherlands, 1986).
6. Wilhelm, K. *et al. Proc. 20th ESLAB Symp.* Vol. II. Heidelberg, ESA SP-250 (eds B. Batrick, E. J. Rolfe & R. Reinhard) 367-369 (European Space Agency, Noordwijk, Netherlands, 1986).
7. Hughes, D. W. *Mon. Not. R. astr. Soc.* **213**, 103-109 (1985).
8. Divine, N. *et al. Space Sci. Rev.* **43**, 1-104 (1986).
9. Bertaux, J. L. & Abergel, A. *Proc. 20th ESLAB Symp.* Vol. II. Heidelberg, ESA SP-250 (eds B. Batrick, E. J. Rolfe & R. Reinhard) 341-345 (European Space Agency, Noordwijk, Netherlands, 1986).
10. Greenberg, J. M. & Grim, R. *Proc. 20th ESLAB Symp.* Vol. II. Heidelberg, ESA SP-250 (eds B. Batrick, E. J. Rolfe & R. Reinhard) 255-263 (European Space Agency, Noordwijk, Netherlands, 1986).
11. Krasnopolsky, V. A. *et al. Nature* **321**, 269-271 (1986).
12. Gringauz, K. I. *et al. Nature* **321**, 282-285 (1986).
13. Krankowsky, D. *et al. Nature* **321**, 326-329 (1986).
14. Festou, M. C. *et al. Nature* **321**, 361-363 (1986).
15. Craven, D. J. *et al. Geophys. Res. Lett.* **13**, 873-877 (1986).
16. Sagdeev, R. Z. *et al. Nature* **321**, 259-262 (1986).
17. Sekanina, Z. & Larson, S. M. *Astr. J.* **89**, 1408-1425 (1984).
18. Grün, E. *et al. Nature* **321**, 144-147 (1986).
19. Kaneda, E. *et al. Nature* **320**, 140-141 (1986).
20. Belton, M. J. *et al. Proc. 20th ESLAB Symp.* Vol. I. Heidelberg, ESA SP-250 (eds B. Batrick, E. J. Rolfe & R. Reinhard) 599-603 (European Space Agency, Noordwijk, Netherlands, 1986).
21. Whipple, F. L. & Sekanina, Z. *Astr. J.* **84**, 1894-1909 (1979).
22. Millis, R. L. & Schleicher, D. G. *Nature* **324**, 646-649 (1986).
23. Sekanina, Z. *Nature* **325**, 326-328 (1987).
24. Smith, B. A. *et al. Nature* **326**, 573-574 (1987).
25. Keller, H. U. *et al. Proc. 20th ESLAB Symp.* Vol. II. Heidelberg, ESA SP-250 (eds B. Batrick, E. J. Rolfe & R. Reinhard) 359-362 (European Space Agency, Noordwijk, Netherlands, 1986).
26. Landau, L. D. & Lifshitz, E. M. *Course of Theoretical Physics*, Vol. I, 3rd edn (Pergamon, Oxford, 1976).
27. Julian, W. J. *Nature* **326**, 57-58 (1987).
28. McDonnell, J. A. M. *et al. Nature* **321**, 338-341 (1986).
29. Sagdeev, R. Z. *et al. Nature* **321**, 259-262 (1986).
30. Sekanina, Z. & Larson, S. M. *Astr. J.* **92**, 462-482 (1986).
31. Marsden, B. G. *et al. Astr. J.* **78**, 211-225 (1973).
32. Morris, C. S. & Green, D. W. *Astr. J.* **87**, 918-923 (1982).
33. Bortle, J. E. & Morris, C. S. *Sky and Telescope* **67**, 9-12 (1984).
34. Weissman, P. R. *Proc. 20th ESLAB Symp.* Vol. III. Heidelberg, ESA SP-250 (eds B. Batrick, E. J. Rolfe & R. Reinhard) 517-522 (European Space Agency, Noordwijk, Netherlands, 1986).

Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*

Melanie G. Lee & Paul Nurse

Cell Cycle Control Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, UK

A human homologue of the cdc2 gene has been cloned by expressing a human cDNA library in fission yeast and selecting for clones that can complement a mutant of cdc2. The predicted protein sequence of the human homologue is very similar to that of the yeast cdc2 gene. These data indicate that elements of the mechanism by which the cell cycle is controlled are likely to be conserved between yeast and humans.

THE *cdc2* gene plays an important role in controlling the cell cycle of the fission yeast *Schizosaccharomyces pombe*^{1,2}. In this article we describe the isolation and characterization of a *cdc2* homologue from human cells. The gene was cloned by expressing a human cDNA library in *S. pombe* and selecting those clones which could complement a mutation in the *cdc2* gene. This method is applicable for isolating other mammalian genes for which mutants in equivalent genes are available in yeast. The human *CDC2* gene has been sequenced, and potentially encodes a protein of the same molecular weight as *S. pombe cdc2* with a 63% identity of amino-acid residues. This functional and structural similarity indicates that elements of cell cycle control are likely to be conserved between yeast and humans, and therefore will probably be found in all eukaryotic cells.

Cell cycle control

The *cdc2* gene encodes a function which is required at the two major control points during the *S. pombe* mitotic cell cycle^{1,2}. The first control is called start and is located in G1 at the point where the cell becomes committed to the mitotic cell cycle³. Once past start, the cell cannot undergo the alternative developmental fate of conjugation until the cycle in progress is completed, and the cell also begins the programme of events which lead to S-phase⁴. The second control is located in G2 and determines when the cell initiates mitosis^{1,2,5,6}. Recessive temperature sensitive lethal *cdc2* mutants which lack *cdc2* function are unable to proceed past either the G1 or G2 control points⁷. Dominant *cdc2* mutants which contain a *cdc2* function with altered regulatory properties traverse G2 more rapidly and initiate mitosis and cell division at a reduced cell size compared with wild type cells⁸. The *cdc2* gene has been shown to encode a phosphoprotein of relative molecular mass 34,000 (*M_r* 34K) which has protein kinase activity⁹. In nutrient-deprived cells the protein is dephosphorylated and has no kinase activity. On readdition of nutrients the protein gains kinase activity and becomes phosphorylated. This suggests that entry into the mitotic cell cycle could be regulated by modulating *cdc2* protein kinase activity, possibly by phosphorylation⁹. Three genes are important in controlling the *cdc2* gene function at the initiation of mitosis; *cdc25*^{10,11} and *nim1*¹² act as activators and *wee1*^{8,13} acts as an inhibitor. A fourth gene *suc1* probably also interacts with *cdc2* gene function^{14,15}.

A gene analogous to *cdc2* is found in the evolutionarily divergent budding yeast, *Saccharomyces cerevisiae*, and is called *CDC28*¹⁶. This gene function is required at start¹⁷ and probably later in the cycle for mitosis¹⁸. It encodes a 36K phosphoprotein with protein kinase activity¹⁹, and has 62% identity in protein sequence to the *cdc2* gene product²⁰. The similarities in structure and function of the *cdc2* and *CDC28* genes indicate that aspects of cell cycle control are conserved in the two yeasts.

Strategy for cloning

Three strategies can be employed to clone human homologues of known yeast genes: 1, low stringency hybridization to detect shared nucleotide sequences; 2, antibody screening of expression libraries to detect shared structural features; 3, complementation of mutants to detect genes with similar functions. We have used the third of these strategies, employing a human cDNA library to select for a gene that can complement a defective *cdc2* function. This approach enables genes to be isolated not because of their structural similarities but because they can provide a similar function^{21,22}. A human cDNA library was used because all mammalian introns cannot be expected to be spliced out in *S. pombe*. Because plasmids do not require an autonomously replicating sequence (ARS) for *S. pombe* transformation²³ and the SV40 early promoter works well in *S. pombe*, we were able to use the excellent full-length Okayama and Berg human cDNA library made in an SV40 expression vector²⁴. This library was co-transformed with another vector containing a selectable marker allowing cells that had taken up plasmid to be tested for their ability to grow in the absence of the *cdc2* gene function. The vector used was pDB262 which transforms *S. pombe* very efficiently and contains the *S. cerevisiae* *LEU2* gene able to complement *S. pombe leu1-32* (ref. 25).

Cloning *CDC2Hs*

The *S. pombe* temperature sensitive mutant strain *cdc2-33 leu1-32* was co-transformed with the plasmid vector pDB262 and the human cDNA library in the Okayama and Berg expression vector. A total of 10⁶ leucine prototrophic transformants were grown for 24 h at the permissive temperature of 29 °C and were then shifted to the restrictive temperature of 36 °C. Five transformants were found to form colonies. All of these were unstable for growth at 36 °C, indicating that the complementing activity was located on a plasmid. Two to four library plasmids were recovered into *E. coli* from each of the original five transformants. Each of these was tested for the ability to complement *cdc2-33* on retransformation with pDB262 back into the *cdc2-33 leu1-32 S. pombe* strain. Two plasmids, pOB231 and pOB245, isolated from two different original yeast transformants, were found to complement the *cdc2-33* mutation. Restriction mapping demonstrated that the plasmids were identical, each containing a similar 2-kilobase (kb) insert. A restriction map of this is shown in Fig. 1a. *Bam*HI sites are located just outside the GC and AT tails in the vector²⁴, and thus a *Bam*HI digest of pOB231/245 removes the cDNA insert together with a small amount of flanking vector sequence. To establish if the other three original yeast transformants contained sequences related to pOB231/245, a Southern blot was made of *Bam*HI digested DNA prepared from the five yeast strains and was probed with

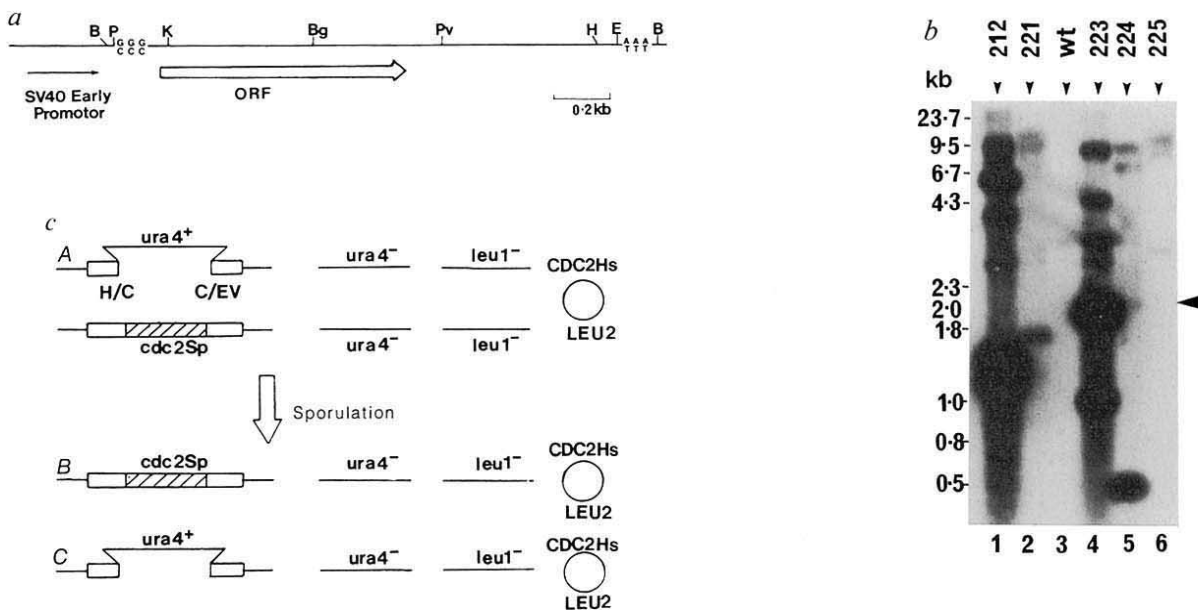


Fig. 1 *a*, Restriction map of the pOB231/245 insert. The restriction sites are B, *Bam*HI; P, *Pst*I; K, *Kpn*I; Bg, *Bgl*II; Pv, *Pvu*II; H, *Hind*III; E, *Eco*RI. The positions of the SV40 early promoter, the sites of the GC and AT tails used in the library construction, and the open reading frame (ORF) are shown. *b*, Southern blot of *Bam*HI-digested DNA made from wild-type cells and the five transformants probed with the cDNA insert of pOB231. Lane 1, strain 212; lane 2, 221; lane 3, wild type; lane 4, 223; lane 5, 224; lane 6, 225. The plasmids pOB231 and pOB245 were derived from strains 223 and 224 respectively. The arrow marks the size of the cDNA insert. *c*, Scheme illustrating the construction of the *cdc2* deletion strain containing pSAB2Hs. *a*, The original diploid strain containing one chromosome homologue with an intact *cdc2* gene (called *cdc2*Sp) showing the ORF as hatched, and a second homologue with the deleted gene and an insertion of *ura4+*. The entire *cdc2*Sp ORF has been deleted between the *Hind*III (H) and *Eco*RV (EV) sites and the *ura4+* gene inserted after addition of *Cla*I (C) linkers. *b*, On sporulating the diploid, half of the spores contained the homologue with the intact *cdc2*Sp gene and thus were uracil auxotrophs. *c*, The other half of the spores contained the homologue deleted for the *cdc2*Sp gene with the *ura4+* insert. Those spores also containing pSAB2Hs formed uracil and leucine prototrophic colonies. *d*, Cells of the *cdc2*Sp deletion strain containing pSAB2Hs. Cells containing the plasmid continue to divide, forming normal sized cells. On plasmid loss the cells become highly elongated and no longer divide.

Methods. *a*, The *S. pombe* strain *cdc2-33 leu1-32* was transformed as described in refs 37 and 38 using 100 μ g of the Okayama Berg cDNA library²⁴ and 100 μ g of pDB262 mixed with 3×10^9 sphaeroplasts. Library plasmids were recovered back into *E. coli* strain JA226, selecting for ampicillin resistance as described in ref. 38. *b*, DNA was prepared from the *S. pombe* strains as described³⁹ *Bam*HI digested DNA was probed with the purified 2-kb *Bam*HI fragment from pOB231 made radioactive by oligolabelling using hexadeoxynucleotide primers⁴⁰. *c*, The gene replacement was carried out using the one step procedure⁴¹ as modified for *S. pombe*¹¹. The plasmid pSAB2Hs containing the pOB231 insert (called CDC2Hs) and *LEU2* was transformed into this diploid.

the insert prepared from pOB231. Hybridizing sequences were detected in all five transformants (Fig. 1*b*, tracks 1, 2, 4, 5, 6) whereas no hybridization was seen in an untransformed strain (Fig. 1*b*, track 3). Considerable plasmid rearrangement appears to have taken place in all five yeast strains. Only the two yeast transformants which yielded pOB231 and 245 (Fig. 1*b*, tracks 4, 5) contained a band corresponding in size to the 2-kb insert found in pOB231/245.

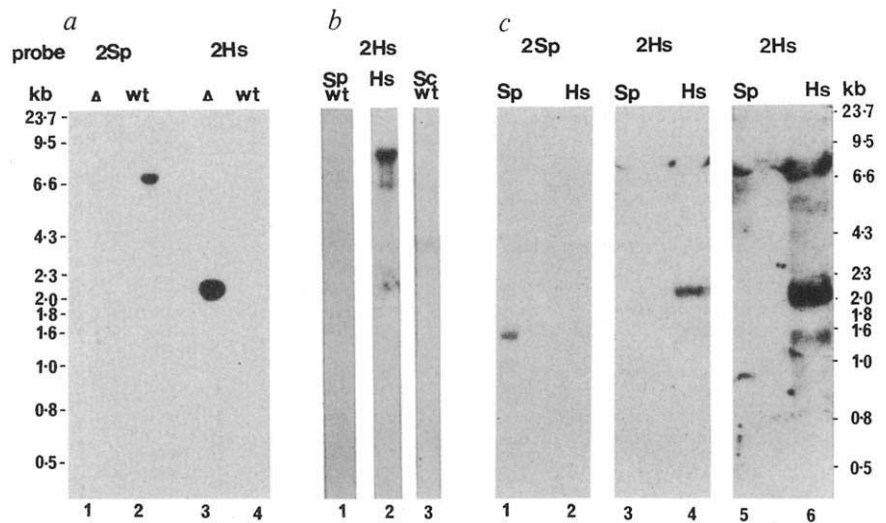
These experiments establish that all of the original yeast transformants contained sequences related to the insert of pOB231 and that this plasmid could complement the *cdc2-33* mutation. Our failure to recover complementary plasmids from the three other yeast transformants was probably due to plasmid rearrangements occurring in yeast which prevented their recovery in *E. coli*.

To prove that the insert in pOB231 could provide all the

functions encoded by *cdc2*, the 2-kb *Bam*HI insert was transferred to the *LEU2* and 2 μ m-containing *S. pombe* plasmid vector pSAB1 (pDB248X²⁵, with a *Sal*I site deleted), to generate plasmid pSAB2Hs. This plasmid complemented all five *cdc2*^{ts} mutants tested, *cdc2-56*, L7, M26, M55 and M63, and also enabled an *S. pombe* strain deleted for *cdc2* to grow normally. The deletion strain was constructed as shown in Fig. 1*c*. Initially a diploid strain was made which contained *cdc2+* on one chromosome homologue, and a deletion of the entire *cdc2* open reading frame with an insertion of the marker *ura4+* on the other homologue. After transformation with pSAB2Hs the diploid was sporulated. Those spores which contained the chromosome deleted for *cdc2* germinated but failed to undergo cell division, forming elongated cells. But if the spores contained pSAB2Hs, cells lacking *cdc2+* could divide like wild-type cells and formed colonies. Because the cells lose the plasmid

Fig. 2 *a*, Southern blots of DNA prepared from a wild-type strain and the *cdc2Sp* deletion strain containing pSAB2Hs. Lane 1, deletion strain probed with *cdc2* (2Sp); lane 2, wild type probed with 2Sp; lane 3, deletion strain probed with pOB231 cDNA insert (2Hs); lane 4, wild-type probed with 2Hs. *Bam*HI digestion generates a 7-kb fragment containing the *cdc2Sp* gene and a 2-kb fragment containing the pOB231 insert. *b*, Southern blots of DNA prepared from *S. pombe*, *H. sapiens* and *S. cerevisiae* probed with pOB231 insert (2Hs). Lane 1, *S. pombe* DNA; lane 2, *H. sapiens* DNA; lane 3, *S. cerevisiae* DNA. No significant hybridization is seen in *S. pombe* or *S. cerevisiae*, whereas a strong band of 8 kb is seen in *H. sapiens*. *c*, Northern blots of RNA prepared from *S. pombe* wild-type cells and *H. sapiens* cell line HT29. Lane 1, *S. pombe* RNA (Sp) probed with *cdc2Sp* (2Sp); lane 2, *H. sapiens* RNA (Hs) probed with 2Sp; lanes 3, 5, *S. pombe* RNA (Sp) probed with pOB231 cDNA insert (2Hs); lanes 4, 6, *H. sapiens* RNA (Hs) probed with 2Hs. Lanes 5, 6 are longer exposures of lanes 3, 4.

Methods. Polyadenylated RNA was prepared from *S. pombe* wild-type cells as described⁴² and probed with purified fragments of the pOB231 cDNA insert and *cdc2Sp* made radioactive by oligolabelling using hexadeoxynucleotide primers⁴⁰.



frequently these colonies constantly produce highly elongated cells which divide no further (Fig. 1d). To establish that cells from these colonies had all their *cdc2* sequences deleted and contained the pOB231 insert, DNA was prepared and compared with DNA from wild type cells on a Southern blot. Probing with the pOB231 insert showed hybridizing sequences in the growing deletion clone but not in wild type (Fig. 2a, tracks 3, 4), whilst probing with *cdc2* showed the gene in wild type but not in the growing deletion strain (Fig. 2a, tracks 1, 2). Therefore the pOB231 human cDNA insert can provide all the functions of *S. pombe cdc2*. We shall call the gene from which this cDNA is derived the human *CDC2* gene. When necessary to avoid ambiguity we will use the suffix Hs (*CDC2*Hs) to indicate its *Homo sapiens* origin and Sp (*cdc2*Sp) when referring to the *S. pombe cdc2* gene.

Human origin of *CDC2*Hs

It was important to demonstrate that *CDC2*Hs was of human origin. To do this DNA was prepared from *H. sapiens*, *S. pombe* and *S. cerevisiae* and digested with *Bam*HI. *S. cerevisiae* was also included in this experiment as it contains the gene *CDC28* which can complement mutants of *S. pombe cdc2*. After preparing Southern blots the DNAs were probed with *CDC2*Hs. No significant hybridization was observed with *S. pombe* or *S. cerevisiae*, but a major band of 8 kb and a minor band of 6 kb were observed with *H. sapiens* (Fig. 2b). From the band intensity the gene does not appear to be dispersed throughout the human genome and is likely to be single or low copy. To establish whether the gene is expressed in human cells polyadenylated RNA was prepared from the human colonic carcinoma cell line HT29, together with *S. pombe* polyadenylated RNA. Both RNAs were Northern blotted and probed with *cdc2Sp* and *CDC2*Hs. A 1.6-kb transcript hybridizing to *cdc2Sp* was seen in *S. pombe* but not in *H. sapiens* (Fig. 2c, tracks 1, 2), whereas a 2.0-kb transcript hybridizing to *CDC2*Hs was seen in *H. sapiens* (Fig. 2c, tracks 4, 6) but not in *S. pombe*, even after over-exposure of the autoradiograph (Fig. 2c, tracks 3, 5). This confirms that *CDC2*Hs is of human origin and is not derived from *S. pombe* or *S. cerevisiae*, and that it is derived from a 2-kb polyadenylated transcript. As this is the length of the cDNA clone isolated it can be assumed that this clone is full length or almost so.

Sequence homology of *CDC2*Hs

The 2-kb *Bam*HI fragment containing *CDC2*Hs was sequenced by subcloning into pTZ18 and pTZ19 (Pharmacia Ltd.,

Molecular Biology Division) using exonuclease III deletion and *Alu*I and *Hae*III digestion. An extensive open reading frame (ORF) was located in the 1-kb fragment passing through the *Kpn*I and *Bgl*II sites. Sequencing both strands in this region confirmed that *CDC2*Hs potentially codes for a protein of 297 amino acids. The nucleotide sequence and its translation are given in Fig. 3.

The predicted ORF shares extensive homology with the fission yeast gene *cdc2Sp* and the budding yeast gene *CDC28* (Fig. 4). Amino-acid identities throughout the proteins are 63% between *CDC2*Hs and *cdc2Sp*²⁰ and 58% between *CDC2*Hs and *CDC28*²⁶. If only those regions which are identical between *cdc2Sp* and *CDC28* are compared with *CDC2*Hs the identity rises to around 80%. The overall length of the three proteins are almost identical at 297 amino acids for *CDC2*Hs, *cdc2Sp* and 298 amino acids for *CDC28*. The predicted ORF of *CDC2*Hs also contains the two consensus regions surrounding the ATP binding (Fig. 3, nucleotides 170–239) and phosphorylation sites (Fig. 3, nucleotides 519–728) found in protein kinases¹ suggesting that it encodes a protein kinase. Despite the conservation at the protein level, there are no stretches longer than 14 nucleotides which are the same between *CDC2*Hs and *cdc2Sp*.

The protein sequences of *CDC2*Hs and *cdc2Sp* are remarkably similar. Comparisons of histone H2A/B and α/β tubulin proteins, which are very closely related proteins, show identities between *S. pombe* and mammalian cells of between 68%–76%^{27–29}, not much greater than the 63% we have observed here. We propose that *CDC2*Hs encodes a functional and structural homologue of the fission yeast *cdc2Sp* gene. The human gene *CDC2*Hs is also likely to be the functional homologue of the budding yeast *CDC28* gene, given its structural similarity to this gene.

A *cdc2Sp* like gene has been isolated from a human HeLa cDNA library using synthetic oligonucleotide probes corresponding to regions of expected similarity based on the sequences of conserved stretches within the catalytic domain of serine kinases³⁰. This cDNA has been partially sequenced and this shows 47% identity in protein sequence to *cdc2Sp*. But it is different from *CDC2*Hs and its overall similarity to *cdc2Sp* is less than that found for *CDC2*Hs.

Human protein of *M_r* 34K

To investigate the *CDC2*Hs gene product in human cells, antibodies were raised against a peptide represented by the single letter amino-acid code EGVSTAIKRELLKE, which

GGGGGG GGGGGGCACT TGGCTTCAAA GCTGGCTCTT GGAATTTAG CGGAGACGAG 56
 Sca1 140
 CGGCTTTATG TAGCTGCGCT GGGGGCGCG CGGAATAATA AGCGGGATC TACCATACCA TTGACTAAT

Kpn1 194
 ATG GAA GAT TAT ACC AAA ATA GAG AAA ATT GGA GAA GGT ACC TAT GGA GTT GTG
 M E D Y T K I E K I G F G T Y G V V

Acc1 248
 TAT AAG GGT AGA CAC AAA ACT ACA GGT CAA GTG GTA GCC ATG AAA AAA ATC AGA
 Y K G R H K T T G Q V V A M K K I R

Sca1 302
 CTA GAA AGT GAA GAG GAA GGG GTT CCT AGT ACT GCA ATT CGG GAA ATT TCT CTA
 I E S E E F G V P S T A I R E I S I

356
 TTA AAG GAA CTT CGT CAT CCA AAT ATA GTC AGT CTT CAG GAT GTG CTT ATT CAG
 I K E I R H P N I V S I Q D V I M Q

410
 GAT TCC AGG TTA TAT CTC ATC TTT GAG TTT CTT TCC ATG GAT CTG AAG AAA TAT
 D S R I Y I J F E F I S M D I K K Y

464
 TTG GAT TCT ATC CCT CCT GGT CAG TAC ATG GAT TCT TCA CTT GTT AAG AAT TAT
 I D S J P P G Q Y M D S I V K S Y

Xba1 518
 TTA TAC CAA ATC CTA CAG GGG ATT GTG TTT TCT CAC TCT AGA AGA GTT CTT CAC
 I Y Q J I Q G I V F C H S R R V I H

572
 AGA GAC TTA AAA CCT CAA AAT CTC TTG ATT GAT GAC AAA GGA ACA ATT AAA CTG
 R D I K P Q N I I I D D K G T I K I

626
 GCT GAT TTT GGC CTT GCC AGA GGT TTT GGA ATA CCT ATC AGA GTA TAT ACA CAT
 A D F G I A R A F G I P I R V Y T H

Hg111 680
 GAG GTA GTA ACA CTC TGG TAC AGA TCT CCA GAA GTA TTG CTG GGG TCA GCT CGT
 E V V T I W Y R S P F V I I G S A R

Hind11 734
 TAC TCA ACT CCA GTT GAC ATT TGG AGT ATA GGC ACC ATA TTT GCT GAA CTA GCA
 Y S T P V D J W S I G T I F A E I A

Bcl1 788
 ACT AAG AAA CCA CTT TTG CAT GGG GAT TCA GAA ATT GAT CAA CTC TTG AGG ATT
 T K K P I F H G D S K I D Q I F R I

842
 TTT AGA GCT TTG GGC ACT CCC AAT AAT GAA GTG TGG CCA GAA GTG GAA TCT TTA
 P R A I G T P P N N E V W P F E V E S I

896
 CAG GAC TAT AAG AAT ACA TTT CCC AAA TGG AAA CCA GGA AGC CTA GCA TCC CAT
 Q D Y K N T P P K W K P G S I A S H

950
 GTC AAA AAC TTG GAT GAA AAT GGC TTG GAT TTG CTC TGG AAA ATG TTA ATC TAT
 V K N I D E N G I D I I S K M I I Y

1004
 GAT CCA GCC AAA CGA ATT TCT GGC AAA ATG GCA CTG AAT CAT CCA TAT TTT AAT
 D P A K R I S G K M A I N H P Y P N

1064
 GAT TTG GAC AAT CAG ATT AAG AAG ATG TAG CTTCTGACA AAAAGTTTCC ATATGTTATG
 I I D N Q I K K M

Fig. 3 DNA sequence and its translation of pOB231 insert. Some 6-base recognition restriction enzyme sites are shown above the sequence. The ORF from the first initiator codon to the stop codon is given.

corresponds to nucleotide positions 264–311 (Fig. 3) and is not conserved in other protein kinases (Fig. 3). The serum detected a protein of 34K relative molecular mass on Western blots of protein prepared from the human colonic carcinoma cell line HT29 (Fig. 5a, track 1; Fig. 5b, track 1). A similar-sized protein was also detected in *S. pombe* cells (Fig. 5b, track 2 and Fig. 5c, track 1). This protein was shown to be the *cdc2Sp* gene product by demonstrating that in a yeast strain overproducing *cdc2Sp* transcript the 34K band is increased in intensity (Fig. 5b, track 3). The serum was also shown to detect the *CDC2Hs* protein by Western blot analysis of protein made from the yeast strain deleted for *cdc2Sp* but containing pSAB2Hs. This strain contained no *cdc2Sp* sequences and so could not contain any *cdc2Sp* protein. A 34K protein of similar size to that seen in human cells was detected in this strain (Fig. 5c, track 3; Fig. 5d, track 1). Cells deleted for *cdc2Sp* and not containing pSAB2Hs generated by germinating spores harbouring the chromosome with the deletion, had no 34K protein detectable by the serum (Fig. 5c, track 2). The anti-peptide serum detected a similar-sized protein

2Hs M E D Y T K I E K I G E G T Y G V V Y K A R H K T T
 2Sp M E N Y Q K V E K I G E G T Y G V V Y K A R H K L S
 28 M S G E L A N Y K K L E K V G E G T Y G V V Y K A L D L R P G Q

2Hs G Q V V A M K K I R L E S E E E G V P S T A I R E I S L L K E
 2Sp G R I V A H K K I R L E D E S E G V P S T A I R E I S L L K E
 28 G Q R V V A L K K I R L E S E D E G V P S T A I R E I S L L K E

2Hs L R H P N I V S L Q D V L M Q D S R L Y L I P E F L S
 2Sp V N D E N N R S N C V R L L D I L H A E S K L Y L V P E F L D
 28 L K D D N I V R L Y D I V H S D A H K L Y L V P E F L D

2Hs M D L K K Y L D S I P P G Q Y M D S S L V K S Y L Y Q I L Q
 2Sp M D L K K Y M D R I S E T G A T S L D P R L V Q K F T Y Q L V N
 28 L D L K R Y M E G I P K D Q P L G A D I V K K F M H Q L C K

2Hs G I V F C H S R R V L H R D L K P Q N L L I D D K G T I K L A D
 2Sp G V N F C H S R R I I H R D L K P Q N L L I D K E G N L K L A D
 28 G I A Y C H S H R I L H R D L K P Q N L L I N K D G N L K L G D

2Hs P O L A R A F G I P I R V Y T H E V V T L W Y R S P E V L L G S
 2Sp P G L A R S F G V P L R N Y T H E I V T L W Y R A P E V L L G S
 28 P G L A R A F G V P L R A Y T H E I V T L W Y R A P E V L L G G

2Hs A R Y S T P V D I W S I G T I F A E L A T K K P L P F H G D S E I
 2Sp R H Y S T G V D I W S V G C I F A E M I R R S P L F P G D S E I
 28 K Q Y S T G V D T W S I G C I F A E M C N R A P I F S G D S E I

2Hs D Q L P R I F R A L G T P N N E V W P F V E S L Q D Y K N T P F
 2Sp D E I F K I F Q V L G T P N E A I W P G V T L L Q D Y K S T P F
 28 D Q I F K I F R V L G T P N E A I W P G D I V Y L P D F K P S P F

2Hs K W K P G S L A S H V K N L D E N G L D L L S K M L I Y D P A K
 2Sp R W K R M D L H K K V F P H G E D A I E L L S A M L V Y D P A H
 28 Q W R K R K D L S Q V V P S L D P R G I D L L D K L L A Y D P I N

2Hs R I S G K M A L N H P Y P N D L D N Q I K K M
 2Sp R I S A K R A L Q Q N Y L R D P H
 28 R I S A R R A A I H P Y P Q E S

Fig. 4 Comparison of the predicted amino-acid sequences of *H. sapiens CDC2* (2Hs), *S. pombe cdc2* (2Sp) and *S. cerevisiae CDC28* (28). Identities between *CDC2Hs* and *cdc2Sp* are boxed. When residues in *CDC28* are also identical to the other two sequences these are also boxed.

in three other human cell lines, J6 T cells, Heb7a HeLa-derived cells and Daudi B cells (Fig. 5d, tracks 2–4). A second serum raised against the carboxy-terminal 99 amino-acid residues of *cdc2Sp* (provided by V. Simanis) also detected a 34K protein in these three cell lines (Fig. 5e, tracks 1–3). We have also detected a 34K protein in untransformed Butler human embryo fibroblast cells using the anti-peptide serum (data not shown). These data suggest that the 34K protein is likely to be the *CDC2Hs* gene product.

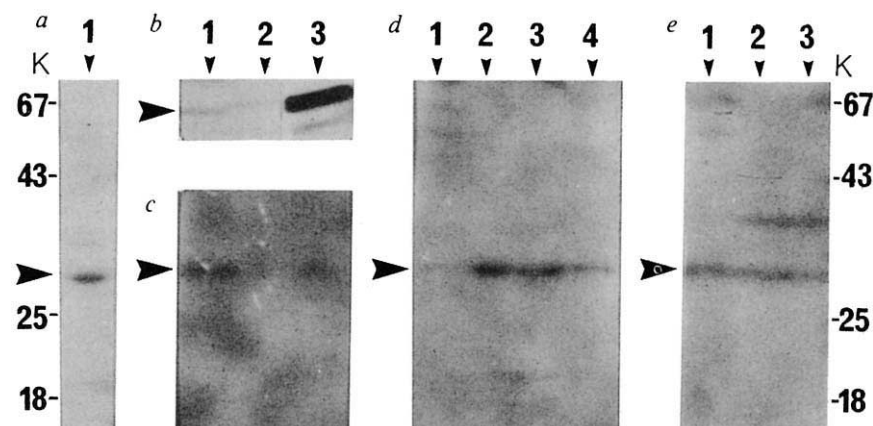
The level of 34K protein produced in the *cdc2Sp* deletion strain containing pSAB2Hs (Fig. 5c, track 3) is similar in level to that produced by *cdc2Sp* in wild-type cells (Fig. 5c, track 1). This suggests that complementation of the fission yeast *cdc2Sp* gene function can be achieved without overproducing the human *CDC2Hs* protein.

Human genes in yeast

The experiments we have described provide an alternative approach for isolating homologous genes to using reduced stringency DNA hybridization or antibodies with a bacterial expression library. For the complementation approach to be successful the cDNA probably must be full length and should be made in a vector that can transform and express the cDNA in yeast. The Okayama and Berg library met these criteria in fission yeast but probably new vectors would have to be developed if other simple eukaryotes such as *S. cerevisiae* or *Aspergillus nidulans* were to be used instead²².

In addition to allowing the isolation of human genes by complementation, *S. pombe* can be used as an experimental system for investigating human gene function. The power of *in vitro* mutagenesis can be coupled with the ease of yeast genetics to isolate mutants in the human gene. Of particular interest are mutants with a dominant phenotype that can be tested for their effects after introduction back into human cells. For example, conditional dominant lethals could be isolated which prevent function of the wild type gene by competing out positive effectors

Fig. 5 Western blot analysis of *S. pombe* and *H. sapiens* protein. Equivalent protein samples loaded: *a*, lane 1, human colonic carcinoma cell line HT29; *b*, lane 1, human colonic carcinoma cell line HT29; lane 2, *S. pombe* wild type; lane 3, *S. pombe*, *cdc2* overproducer; *c*, lane 1, *S. pombe* wild type; lane 2, *S. pombe* germinated spores deleted for *cdc2Sp*; lane 3, *S. pombe* deleted for *cdc2Sp* containing pSAB2Hs. *d*, lane 1, *S. pombe* deleted for *cdc2Sp* containing pSAB2Hs; lane 2, human J6 T cell; lane 3, human Heb7a, HeLa-derived; lane 4, human Daudi B cell; *e*, lane 1, human J6 T cell; lane 2, human Heb7a, HeLa-derived; lane 3, Human Daudi B cell. Blots *a*, *b*, *c*, *d* were made using affinity purified antibodies against the peptide EGVSTAIKRELLKE and *e* using serum against the carboxy-terminal 99 amino-acid residues of *cdc2Sp*. The arrowhead marks the position of the 34K protein.



Methods. Extraction of proteins and preparation of peptides conjugates for rabbit immunization were as described in ref. 9. Western blot procedures were performed using GeneScreen and GeneScreen Plus membranes according to the manufacturer's (NEN) instructions.

or substrates. After isolation in yeast these could be used for knocking out gene function in mammalian cells providing an alternative procedure to present techniques such as antibody injection or anti-sense message.

Cell cycle implications

Because the human *CDC2Hs* gene can provide all of the functions of *cdc2Sp* in fission yeast it is reasonable to assume that it performs a similar role to *cdc2Sp* in controlling the human cell cycle. This conclusion is supported by the structural similarity between the two genes both in overall homology and size of the proteins. But it will be important to establish that *CDC2Hs* does have this role in human cells.

The most likely points of action of *CDC2Hs* during the human cell are cycle, analogous to start and the mitotic control in *S. pombe*, in late G1 at the R-point and in late G2 at the initiation of mitosis (Fig. 6). At the R-point, cells undergo a transition which leads to the cell becoming less dependent on the presence of growth factors and the maintenance of high rates of protein synthesis for completion of the cell cycle³¹⁻³³. This commitment to the mitotic cell cycle could correspond to the passage of start in the yeasts. Initiation of mitosis has been shown to involve maturation promotion factor (MPF) in vertebrate eggs³⁴, and similar activities have been recovered from mammalian mitotic cells³⁵ and the budding yeast³⁶. The action of MPF is quite analogous to the mitotic control which operates in *S. pombe*.

Given the homology of *CDC2Hs* to protein kinases in general and to the *cdc2Sp* protein kinase specifically it is likely that passage through the two control points in the human cell cycle will involve protein phosphorylation. The identification of a

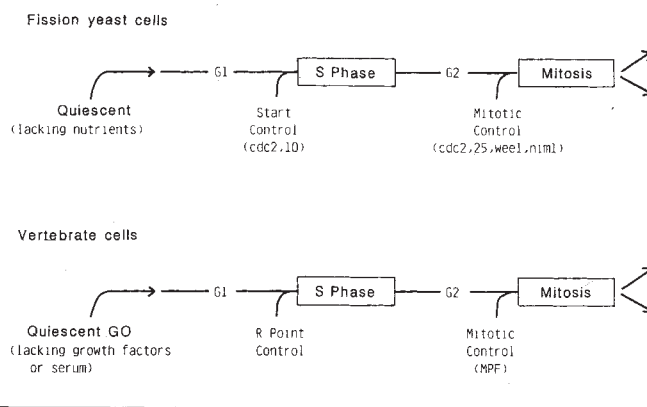


Fig. 6 Comparison of cell cycle control in fission yeast and vertebrate cells. The *S. pombe* start commitment control is analogous to the vertebrate R-point control, and the *S. pombe* mitotic control to the G2/M transition regulated by MPF in vertebrate cells.

cdc2-like function in human cells suggests that elements of the mechanism by which the cell cycle is controlled will probably be found in all eukaryotic cells.

We thank Martin Goss for technical assistance, Viesturs Simanis for antiserum, RNA, and DNA constructs, and other members of the laboratory for helpful discussions; Lionel Crawford for human DNA; Jonathan Rothbard for peptide synthesis; Marianne Dieckmann for supplying the Okayama and Berg library and Clare Middlemiss for typing this manuscript.

Received 18 March; accepted 6 April 1987.

- Nurse, P. *Trends Genet.* **1**, 51-55 (1985).
- Hayles, J. & Nurse, P. *J. cell. Sci. Suppl.* **4**, 155-170 (1986).
- Nurse, P. & Bissett, Y. *Nature* **292**, 558-560 (1981).
- Matsumoto, S., Yanagida, M. & Nurse, P. *EMBO J.* (in the press).
- Nurse, P. *Nature* **256**, 547-551 (1975).
- Thuriaux, P., Nurse, P. & Carter, B. *Molec. Gen. Genet.* **161**, 215-220 (1978).
- Nurse, P., Thuriaux, P. & Nasmyth, K. *Molec. Gen. Genet.* **146**, 167-178 (1976).
- Nurse, P. & Thuriaux, P. *Genetics* **96**, 627-637 (1980).
- Simanis, V. & Nurse, P. *Cell* **45**, 261-268 (1986).
- Fantes, P. *Nature* **279**, 428-430 (1979).
- Russell, P. & Nurse, P. *Cell* **45**, 145-153 (1986).
- Russell, P. & Nurse, P. *Cell* (in the press).
- Russell, P. & Nurse, P. *Cell* (in the press).
- Hayles, J., Aves, S. & Nurse, P. *EMBO J.* **5**, 3373-3379 (1986).
- Hindley, J., Phear, G., Stein, M. & Beach, D. *Molec. Cell. Biol.* **7**, 504-511 (1987).
- Beach, D., Durkacz, B. & Nurse, P. *Nature* **300**, 706-709 (1982).
- Pringle, J. & Hartwell, L. in *The Molecular Biology of the Yeast Saccharomyces—Life Cycle and Inheritance* (eds Strathern, J., Jones, E. & Broach, J.) 97-142 (CSH, New York, 1981).
- Piggott, J., Rai, R. & Carter, B. *Nature* **298**, 391-394 (1982).
- Reed, S., Hadwiger, J. & Lorincz, A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4055-4059 (1985).
- Hindley, J. & Phear, G. *Gene* **31**, 129-134 (1984).

- Henikoff, S., Tatchell, K., Hall, B. D. & Nasmyth, K. *Nature* **289**, 33-37 (1981).
- McKnight, G. L. *et al.* *EMBO J.* **4**, 2093-2099 (1985).
- Heyer, W. D., Sipiczki, M. & Kohli, J. *Molec. Cell. Biol.* **6**, 80-89 (1986).
- Okayama, H. & Berg, P. *Molec. Cell. Biol.* **3**, 280-289 (1983).
- Wright, A., Maundrell, K., Heyer, W. D., Beach, D. & Nurse, P. *Plasmid* **15**, 156-158 (1986).
- Lorincz, A. & Reed, S. *Nature* **307**, 183-185 (1984).
- Hiraoka, Y., Toda, T. & Yanagida, M. *Cell* **39**, 349-358 (1984).
- Toda, T., Adachi, Y., Hiraoka, Y. & Yanagida, M. *Cell* **37**, 233-242 (1984).
- Matsumoto, S. & Yanagida, M. *EMBO J.* **4**, 3531-3538 (1985).
- Hanks, S. K. *Proc. natn. Acad. Sci. U.S.A.* **84**, 388-392 (1987).
- Pardee, A. B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1286-1290 (1974).
- Pardee, A. B., Dunbrow, R., Hamlin, J. L. & Kletzien, R. F. *A. Rev. Biochem.* **47**, 715-750 (1978).
- Zetterberg, A. & Larsson, O. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5365-5369 (1985).
- Kirschner, M., Newport, J. & Gerhart, J. *Trends Genet.* **1**, 41-47 (1985).
- Nelkin, B., Nichols, C. & Vogelstein, B. *FEBS Lett.* **109**, 233-238 (1980).
- Weintraub, H. *et al.* *C. r. heb. Séanc. Acad. Sci. Paris Ser. II*, **295**, 787-790 (1982).
- Beach, D. & Nurse, P. *Nature* **290**, 140-142 (1981).
- Beach, D., Piper, M. & Nurse, P. *Molec. Gen. Genet.* **187**, 326-329 (1982).
- Durkacz, B., Beach, D., Hayles, J. & Nurse, P. *Molec. Gen. Genet.* **201**, 543-545 (1985).
- Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **132**, 6-13 (1983).
- Rothstein, R. J. *Meth. Enzymol.* **101**, 202-211 (1983).
- Aves, S., Durkacz, B., Carr, A. & Nurse, P. *EMBO J.* **4**, 457-463 (1985).

Possible binary star progenitor for SN1987A

Graeme L. White* & D. F. Malin†

* Division of Radiophysics, CSIRO, PO Box 76, Epping, New South Wales, 2121, Australia

† Anglo-Australian Observatory, PO Box 296, Epping, New South Wales, 2121, Australia

Accurate optical astrometry gives a position (B1950.0) for the Large Magellanic Cloud supernova, SN1987A, relative to the FK4 system as right ascension, $RA = 05^h 35^m 49.95 \pm 0.039^s$ ($0.21''$), declination $\delta -69^\circ 17' 57.9'' \pm 0.27''$. Differential astrometry carried out on prime-focus plates taken with the Anglo-Australian telescope indicates that the component, star 1, of Sanduleak's star Sk-69202 is within 0.05 ± 0.13 arc s of the supernova. Here we report that this precision astrometry, together with estimates of the probability of a chance association with a foreground or background star, lead us to conclude that the progenitor of SN1987A was star 1 or a fainter binary companion.

Many photographs of the Large Magellanic Cloud (LMC) region containing both the bright complex nebular region 30 Doradus and the progenitor of supernova SN1987A have been taken with the prime-focus camera of the Anglo-Australian telescope (AAT).

Details of AAT plates referred to here are given in Table 1. Plates 1 and 2 were taken before the supernova event. Plates 3 and 4 are 15 s and 15 min exposures of SN1987A obtained on 1987 February 27.4 (UT). The astrometric plates are 2 and 3. Plate 3 was taken on the same centre as previous fields of 30 Doradus using a red-sensitive emulsion to match the characteristics of the astrometric plate 2, which was taken on 1984 February 05. Plate 4 shows how the supernova now dominates the whole region.

The determination of accurate positions in the far southern sky is not easy because of the paucity of precise reference standards. For our determination of the position of SN1987A we have used stars from the Perth 70 Catalogue¹ as primary standards to establish the positions of 10 transfer standards of ~ 12 mag for use on plate 3. All plate measurements were made using the Bolton x - y measuring machine at the Anglo-Australian Observatory and reduction carried out using the STARLINK software CHART and ASTROM. Thirteen Perth stars within 3.5° of the supernova, and the transfer standards, were measured on Schmidt fields 56 and 57 of both film copies of the SERC SR Sky Atlas and glass copies of the ESO B Sky Atlas. Nine Perth stars were measured on field 56 and eleven on field 57. The SERC J Sky Atlas was not used because the nebulosity associated with the image of 30 Doradus extends over the progenitor and the transfer standards. Each Schmidt field was measured twice, with the orientation of the plate differing by 180° to eliminate systematic errors. The standard errors in the positions of each transfer standard was 0.20 arc s

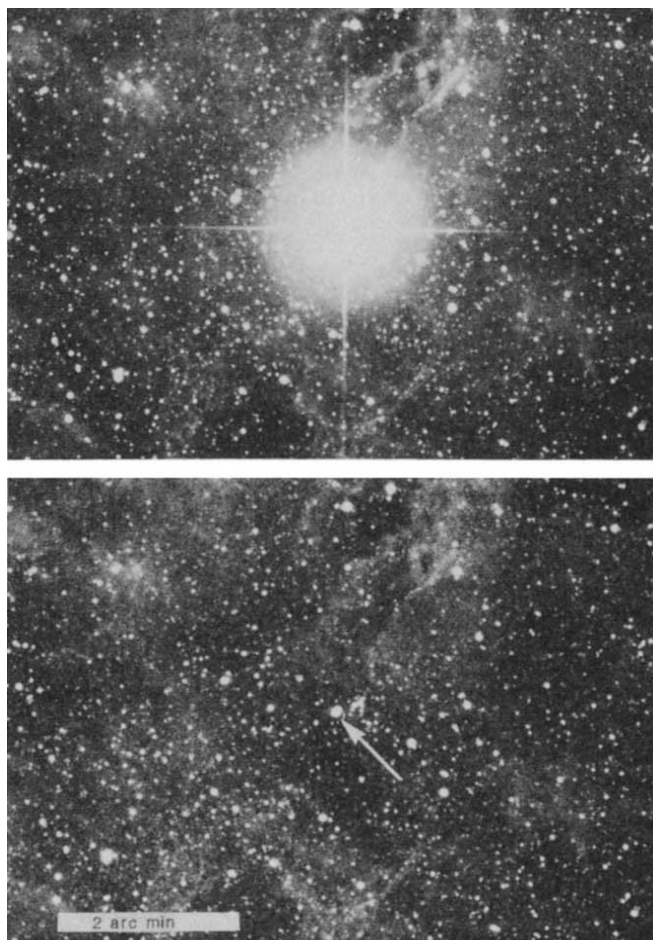


Fig. 1 Enlargements of AAT prime-focus plate 1 and 4 (Table 1) showing the LMC supernova, SN1987A, and the progenitor field. The scale bar is 2 arc min long.

and 0.26 arc s in right ascension and declination respectively. There is no significant offset in the positions of the transfer stars as determined from the red (SR) and blue (B) Schmidt plates. Offsets might have resulted from either differential atmospheric refraction or the effect of the star colours. The mean difference in position (SR - B) is 0.2 ± 0.4 arc s and 0.4 ± 0.6 arc s in right ascension and declination respectively.

AAT plate 3 was measured four times with the plate orientation differing by 90° . Relative to the transfer stars the position of the supernova was determined as $05^h 35^m 49.95 \pm 0.039^s$ ($0.21''$), $-69^\circ 17' 57.9'' \pm 0.27''$ for equinox B1950.0 (epoch 1976.9)². This position precesses to $05^h 35^m 27.99^s$, $-69^\circ 16' 11.5''$ at equinox J2000.0. The errors are dominated by the uncertainties in the positions of the transfer stars. For the purpose of comparison with future accurate radio positions it is noted (G.L.W. *et al.*, in preparation) that the FK4 positions determined with respect to stars from the Perth catalogue are within 0.2 arc s in both coordinates, of the Southern Hemisphere radio reference frame.

Figure 1 has been prepared from the AAT plates 1 and 4 to show the appearance of the field before and after the supernova event. Plates 1 and 2 show Sk-69202 as a double with overlapping image. The progenitor field is also shown schematically in Fig. 2. The existence of a third close companion (star 3) was suspected from the original of AAT plate 2 and has subsequently been confirmed^{3,4}.

Differential astrometry has been undertaken on plates 2 and 3 in order to identify the most likely progenitor. Table 2 gives the positions of the transfer stars determined from AAT plate

Table 1 AAT plate data

	Plate 1	Plate 2	Plate 3	Plate 4
Date	1983 Jan. 18	1984 Feb. 5	1987 Feb. 27.4	1987 Feb. 27.4
Exposure	15 min	35 min	0.25 min	15 min
Centre	30 Doradus	30 Doradus	30 Doradus	SN1987A
Seeing	2 arc s	1.5 arc s	2 arc s	2 arc s
Emulsion	098-04	098-04	IIIa F	IIIa F
Corrector	Gascoigne Triplet for all plates ($15.3 \text{ arc s mm}^{-1}$)			
Filter	RG610 for all plates			

Plates 2 and 3 are astrometric.

Plates 1 and 2 are pre-supernova.

30 Doradus centre is $05^h 38^m 30.0^s$, $-69^\circ 16' 01''$ (B1950.0).
SN1987A centre is $05^h 35^m 49.8^s$, $-69^\circ 17' 57''$ (B1950.0).

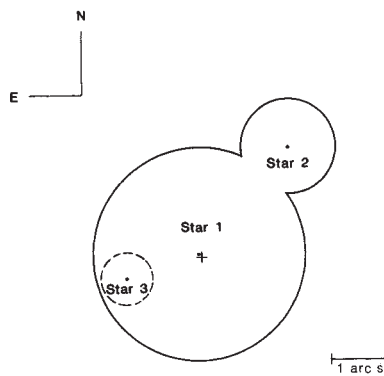


Fig. 2 A schematic of the progenitor field of SN1987A. The relative position of star 3 is from Chu⁴. The separation between star 1 and star 2 is 2.65 ± 0.13 arc s at PA 320° . The cross near the centre of star 1 is the relative position of the supernova; the uncertainty is shown by the length of the bars.

Table 2 Positions of transfer standards from AAT Plate 3

No.	RA (B1950.0) h min s s	Dec. (1950.0)
1	05 41 41.046 ± 0.011	$-69^\circ 06' 28.21'' \pm 0.06''$
2	05 40 47.441 (0".06)	$-69^\circ 16' 01.24''$
3	05 41 22.178	$-69^\circ 21' 57.80''$
4	05 36 27.762	$-69^\circ 25' 52.18''$
5	05 37 03.972	$-69^\circ 10' 34.86''$
6	05 34 37.789	$-69^\circ 15' 07.51''$
7	05 36 03.823	$-69^\circ 04' 25.90''$
8	05 38 08.027	$-68^\circ 56' 38.10''$
9	05 40 42.141	$-69^\circ 32' 22.74''$
10	05 41 04.036	$-68^\circ 57' 51.75''$

Relative to the Perth reference system position uncertainties are 0.20 arc s and 0.26 arc s in right ascension and declination respectively. Star 6 has a high proper motion in declination and was not used.

2 with respect to the Schmidt positions. The uncertainties in these relative positions are 0.06 arc s in both coordinates. Their uncertainty with respect to the Perth reference frame is 0.20 arc s in right ascension and 0.26 arc s in declination. Measurement of plates 2 and 3 relative to the positions of Table 2 show excellent coincidence between the position of the supernova and star 1, the brighter component (south following), of Sk-69202. The positional difference (SN1987A-star 1) is 0.03 arc s ± 0.09 arc s and 0.04 arc s ± 0.10 arc s in right ascension and declination respectively². The position of the supernova and its uncertainty relative to the progenitor field are shown in

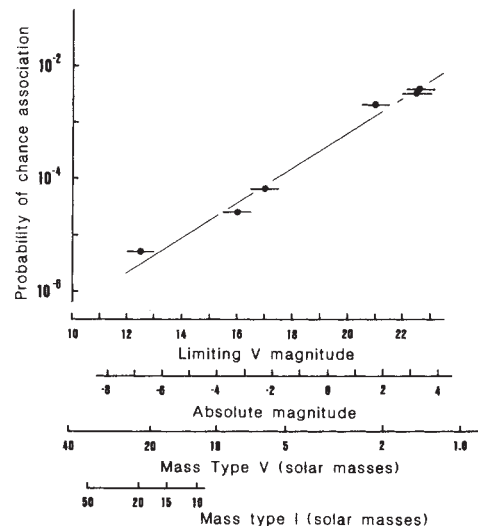


Fig. 3 The probability of a chance occurrence of a star within the 0.113 square arc s error region defined by the precision differential astrometry, as a function of limiting magnitude. Additional ordinates give the corresponding absolute magnitudes and estimated masses for stars of Type I and V.

Fig. 2. These data strengthen the identification proposed by West⁵. Star 2 is 2.65 ± 0.13 arc s removed at position angle (PA) 320° and cannot be considered the identification. The relative position of star 3, at 1.4 arc s, PA 110° (star 1 to 3)⁴, excludes the identification of that star as the progenitor of SN1987A.

Table 3 gives the observed and inferred properties of Sk-69 202. The magnitude estimate of star 1 of $m_V = 12.6$ assumes that star 2 is 1 mag fainter than star 1. With a distance modulus of 18.5 for the LMC⁶ and ~ 0.8 mag for absorption⁷, the absolute magnitude of star 1 is $M_V = -6.7$, typical of a Type Ia supergiant⁸ and the inferred mass is $\sim 30 M_\odot$ ⁸. Trimble⁹ has summarized various studies which suggest that progenitors of Type II supernovae are population I stars of mass greater than $7 \pm 3 M_\odot$.

However, despite the above excellent positional agreement star 1 may not be the progenitor, since observations from the International Ultraviolet Explorer (IUE) satellite¹⁰ suggest the presence of an early-type star (presumably star 1) in the field some days after the supernova event. To show conclusively that the progenitor is star 1, or a previously unseen binary companion of star 1, it is necessary to investigate the possibility of the progenitor being either a foreground or background star coincident with star 1 only by chance. Figure 3 gives the probability

Table 3 Observed and inferred values for Sk-69 202

Parameter	This work	Other references
Magnitudes: m_{pg}		12.2 (ref. 15)
m_V		12.24 (ref. 16)
$B-V$		0.04 (ref. 16)
$U-B$		-0.65 (ref. 16)
No. of components	2, possibly 3	2 (ref. 5), 3 (refs 3, 4)
Separation: star 1 to 2	2.65 arc s ± 0.13 arc s, PA 320°	$< 1''$, PA 110° (ref. 3)
star 1 to 3		$1.4''^{+0.1''}_{-0.2''}$, PA $110^\circ \pm 3^\circ$ (ref. 4)
Magnitude difference, 1-2	~ 1.5 mag in R	~ 1 mag in U; ~ 3 mag in R (ref. 5)
Spectral type		OB (ref. 15); B3 (ref. 16)
Corrected magnitude, m_V	12.6	
Absolute magnitude, M_V	-6.7	
Luminosity class	Ia	
Inferred mass	$\sim 30 M_\odot$	I (ref. 16)

of a chance association within the measured 2σ error ellipse (0.113 square arc s) as a function of limiting magnitude. The probabilities have been computed from star densities observed within ~ 30 arc min of the progenitor on plates 1 and 2 and various photographic surveys¹¹⁻¹⁴. Additional ordinates give the absolute magnitude (calculated using the above distance modulus and absorption) and mass of any progenitor of Type I and V stars⁸. For all observable magnitude regions, and for all acceptable progenitor masses, the probability of a chance association is vanishingly small at $<10^{-4}$.

We conclude, therefore, that the progenitor of SN1987A was not a chance positional coincidence with star 1, but either star 1 itself, or a companion star that was fainter by at least 1 mag and hence not readily discernible in the pre-event photometric, astrometric and spectroscopic observations of Sk-69 202. Clearly observations of all objects in the field will be crucial after the supernova has faded in order to confirm that star 1 was not the progenitor, to establish pre-event properties with more precision, and to ascertain whether star 1 displays peculiar properties as might be expected if its companion were the supernova.

We thank the Director of the Anglo-Australian Observatory for rescheduling the AAT at short notice to secure post-event plates, and Steven Lee for obtaining those plates.

Received 2 April; accepted 10 April 1987.

1. Höp, E. & von der Heide, J. *Abh. Hamburger Sternw.* Vol. IX (1976).
2. White, G. L. & Malin, D. F. *IAU Circ.* No. 4327 (1987).
3. Walborn, N. R., Lasker, B. M., McLean, B. & Laidler, V. G. *IAU Circ.* No. 4321 (1987).
4. Chu, Y.-H. *IAU Circ.* No. 4322 (1987).
5. West, R. M. *IAU Circ.* 4319 (1987).
6. Feast, W. M. S. *Afr. Obs. Circ.* No. 397 (1986).
7. Shelton, I. & Madore, B. *IAU Circ.* No. 4330 (1987).
8. Allen, C. W. *Astrophysical Quantities* 3rd edn (Athlone, London, 1973).
9. Trimble, V. *Rev. mod. Phys.* **54**, 1183-1224 (1982).
10. Sonneborn, G. & Kirshner, R. *IAU Circ.* No. 4333 (1987).
11. Doughty, N. A., Shane, C. D. & Wood, F. B. *The Mount John University Observatory Photographic Sky Survey and Canterbury Sky Atlas (Australis)* (Department of Physics, University of Canterbury, Christchurch, 1972).
12. Hodge, P. W. & Wright, F. W. *The Large Magellanic Cloud* (Smithsonian Press, Washington D.C., 1967).
13. White, G. L. *Proc. astr. Soc. Austr.* **3**, 128-129 (1977).
14. SERC J. Sky Atlas.
15. Sanduleak, N. *Cerro-Tolo Inter-American Obs. Contr.* No. 89 (1969).
16. Rousseau, J. *et al. Astr. Astrophys. Suppl.* **31**, 243-260 (1978).

A prompt radio burst from supernova 1987A in the Large Magellanic Cloud

A. J. Turtle*, D. Campbell-Wilson*, J. D. Bunton†, D. L. Jauncey‡, M. J. Kesteven‡, R. N. Manchester‡, R. P. Norris‡, M. C. Storey‡ & J. E. Reynolds§

* School of Physics, University of Sydney, New South Wales, 2006, Australia

† Department of Electrical Engineering, University of Sydney, New South Wales, 2006, Australia

‡ Division of Radiophysics, CSIRO, P.O. Box 76, Epping, New South Wales, 2121, Australia

§ Mount Stromlo Observatory, Private Bag, Woden, Australian Capital Territory, 2606, Australia

Supernova 1987A in the Large Magellanic Cloud was discovered¹ on 24 February 1987; optical observations define the geocentric time of the explosion to be between February 23.10 and February 23.443 UT^{2,3}. The supernova rose quickly to a visual magnitude of about 4.2 and has remained near that level since. Optical spectral line observations⁴ show that the photosphere was initially expanding at $\sim 16,000$ km s⁻¹ and that the supernova is probably of Type II, that is, from a massive progenitor star. Its position is coincident with a 12th magnitude blue star⁵ but International Ultraviolet Explorer (IUE) observations⁶ suggest that this blue star may still be present. The supernova progenitor star may therefore have been a fainter companion to this star. Radio emission from the supernova was detected at Australian observatories within two days of the increase in optical brightness. Here we report information obtained at four frequencies, namely 0.843, 1.415, 2.29 and 8.41 GHz. At frequencies around 1 GHz the peak flux density was about 150 mJy and occurred within four days of the supernova. The event may be a small precursor to a major flare of the type previously observed in radio supernova.

Observations at 0.843 GHz were made with right-hand circular polarization using the Molonglo Observatory synthesis telescope (MOST)⁷. After a twelve-hour observation this telescope produces a radio map of a field 70 arc min in diameter with an angular resolution of 45 arc s. A field containing the position of the supernova had been observed on 27 December 1986 as part of a general survey of the Large Magellanic Cloud and showed several known supernova remnants, H II regions (including the brightest feature on the map, 30 Doradus) and numerous background sources⁸. Most of the observations of the supernova

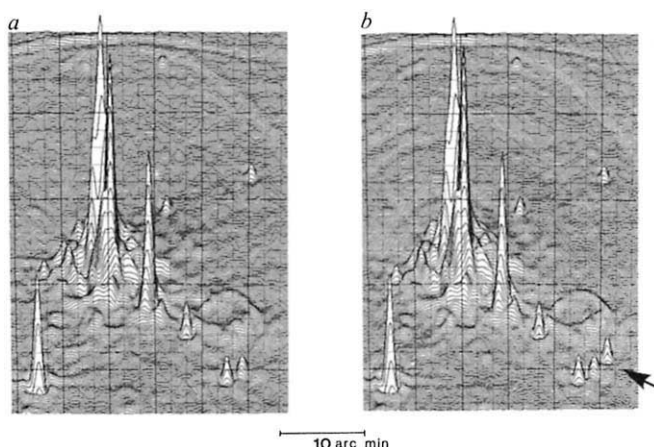


Fig. 1 Representations of the MOST maps of the radio emission at 0.843 GHz for the region of the Large Magellanic Cloud supernova a, before the supernova on 27 December 1986, and b, after the supernova on 26 February 1987. The supernova source is arrowed.

were made with the same field centre as this earlier map and its subtraction left only the supernova amid low-level residuals arising from minor variations in telescope performance. This procedure minimized the difficulties of reliable measurement which arise in the neighbourhood of a strong source like 30 Doradus. A flux density of 13.76 Jy for the source 1934-638 defined the flux density scale and the r.m.s. fluctuation in the difference maps was 2 mJy. Figure 1 shows maps of the region before and after the supernova.

Observations at 1.415 GHz were made with the array of six 13.7-m dishes from the Fleurs synthesis telescope (FST)⁹ with east-west linear polarization. After an eight-hour observation and subsequent cleaning a map of a field of 66 arc min was produced with a resolution of 25 arc s. Estimates of flux density were then made at the position of the supernova. The flux scale was again defined by 1934-638 which was assumed to have a flux density of 16.4 Jy at this frequency. The r.m.s. uncertainty is estimated as 10 mJy, larger than usual because of the combined effect of limited spatial frequency coverage by the six-dish array and the proximity of 30 Doradus.

Observations at higher frequencies were made with the Parkes-Tidbinbilla interferometer (PTI)¹⁰ which comprises the 64-m telescope at Parkes New South Wales and, during this period, one of the 34-m NASA antennas at Tidbinbilla near

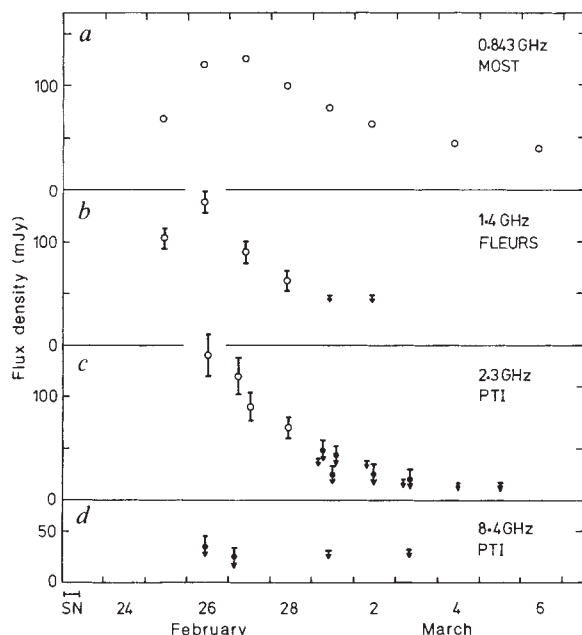


Fig. 2 Time dependence of the radio flux density of supernova 1987A at four frequencies. Definite detections are indicated by a circle, marginal detections by a dot and upper limits in the usual way. Uncertainties due to random errors are indicated; for the MOST data they are smaller than the circle. The instrument used at each frequency is indicated. *a*, 0.843 GHz, MOST; *b*, 1.4 GHz, Fleurs; *c*, 2.3 GHz PTI and *d*, 8.4 GHz PTI.

Canberra. The PTI uses a radio link to send the 10-MHz bandwidth signals from Tidbinbilla over the 275-km baseline to Parkes where they are correlated in real time with the Parkes signals. The interferometer was used with right-hand circular polarization at 2.29 and 8.41 GHz where it has an angular resolution of 0.09 and 0.03 arc s respectively. The strong nearby sources are totally resolved by the interferometer thereby eliminating the confusion problem. Calibration was based on single-dish observations of Hydra A, assumed to have flux densities of 27.42 and 7.99 Jy at 2.29 and 8.41 GHz respectively, and 0537-441, assumed to be unresolved by the interferometer. The derived flux densities for 0537-441 at the time of the observations were 4.8 and 6.1 Jy at the two frequencies.

Results of observations in the period 25 February to 6 March are shown in Fig. 2. At 0.843 GHz the source reached a peak flux density at about February 27.0. After the peak, the flux at 0.843 GHz dropped with an initial half life of 3.0 days, but the subsequent rate of decrease was smaller. At 1.4 GHz the source peaked at about February 26.0, and at 2.3 GHz the peak was not observed but occurred on or before February 26.4. At 8.4 GHz a marginal detection was achieved near the limit of the instrument on the first two days of observation and no peak was observed.

The spectrum near the time of peak emission at 1.4 GHz is shown in Fig. 3. At this time the spectrum was approximately flat at frequencies below 3 GHz, but at higher frequencies the spectral index is -1 or steeper. At later times the spectral peak moved to lower frequencies. Limits on the angular size of the source from the PTI measurements imply that its brightness temperature was greater than 10^7 K at 2.3 GHz and hence that the emission is non-thermal. At all frequencies the measured position is within a few arcs of the optical position given by White and Malin⁵, that is, right ascension (B1950) = 05 h 35 min 49.95 s, dec. (B1950) = $-69^\circ 17' 57.9''$.

If the supernova is at a distance of 50 kpc and radiates isotropically, the peak recorded flux density of about 130 mJy extending over a bandwidth of about 5 GHz implies a radio

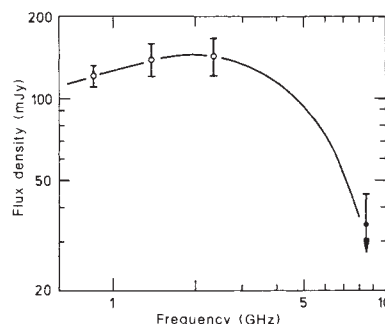


Fig. 3 Spectrum of the radio emission on 26 February 1987, the day of peak emission at 1.4 GHz. Error bars include a contribution from the estimated scale uncertainty at each frequency.

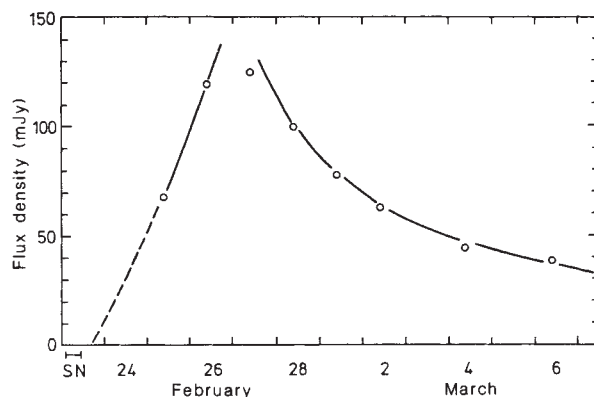


Fig. 4 Fit of a model to the time evolution of the MOST 0.843 GHz data. The model is an expanding shell in which there is both emission of synchrotron radiation and free-free absorption with parameters $m = 0.92$ and $\gamma = 3$ (see text) and a zero point in time of February 23.7. For the rising part of the curve the plasma is optically thick and in the falling part it is optically thin.

luminosity of 2×10^{26} W. Over the five days of the burst the energy radiated at radio wavelength is 8×10^{31} J. This is about 10^{13} times as energetic as a typical solar radio burst, but is minute compared to the 10^{44} J released in a supernova explosion. The peak luminosity of 4×10^{16} W Hz^{-1} is also much less than the peak value at 5 GHz deduced¹¹ for the Type II supernovae SN 1979C in NGC4321 (3×10^{20} W Hz^{-1} after 900 days) and SN1980K in NGC6946 (10^{19} W Hz^{-1} after 140 days). A burst similar to that reported here could not have been detected in any previous observation of an extragalactic supernova. It is perhaps significant that the observed burst, including its time and spectral evolution and its luminosity, is similar to the radio bursts observed from Circinus X-1¹².

Compared to previously observed radio bursts from Type II supernovae the peak luminosity at frequencies of a few GHz is lower by a factor of about 10^3 and the timescales of both the rise and the fall of the radio radiation are about two orders of magnitude shorter. These differences indicate that the existing models devised to explain radio radiation from supernova explosions are not directly applicable to the prompt radio burst from SN1987A.

The observed time and spectral variations suggest a model in which the radio radiation is synchrotron emission by relativistic electrons embedded in an expanding free-free absorbing plasma. Initially, the observed flux density rises because the expansion decreases the optical depth of the absorbing plasma. In the optically thin limit, the decrease in the relativistic electron and magnetic field energy densities results in a decrease in the source luminosity with time. Chevalier¹³ has shown that the time dependence of the observed flux density is $t^{(18m-\gamma-11)/2}$ in the optically

thick limit and $t^{-(\gamma+5-6m)/2}$ in the optically thin limit, where γ is the power-law index of the electron energy distribution, m is defined by $R \propto t^m$, R is the shell radius and t is the time since the supernova explosion. For $m \sim 0.92$ and $\gamma \sim 3$, these functions give a good fit to the 0.843 GHz data as shown in Fig. 4. For the parameters chosen, the optically thin spectrum has a spectral index of -1 which agrees well with the high-frequency end of the spectrum presented in Fig. 3. An external absorbing screen, invoked^{11,13} to account for previous observations of radio emission from Type II supernovae, does not give such a good fit to the data.

We suggest two possible locations for the emitting and absorbing plasma: (1), in a relatively thin shell associated with the expanding supernova shock and (2), in a circumstellar shell of radius about a light-day ejected by the supernova star during its earlier evolution. In this latter case, the observed radio burst must be attributed to synchrotron electrons generated in the outer part of this shell within hours of the explosion. We suggest that relativistic electrons were created within the shell by a pair production cascade from a high-energy γ -ray burst emitted from the stellar core at the time of the supernova. If the progenitor was a highly-evolved core star, the γ -ray burst could escape from the star and pair-production would occur in the circumstellar shell. The possible detection of positronium lines in IUE spectra^{14,15} of the supernova supports the idea of pair production in this region. A single high-energy photon will produce an electron energy distribution with $\gamma = 2$ (ref. 16) but with a range of incident γ -ray energies and electron scattering, the electron spectrum could be softer, so that $\gamma \approx 3$ is plausible. Because essentially all of the γ -ray energy is converted to relativistic electrons, the total energy of the γ -rays which penetrate to the outer part of the absorbing shell need only be a minute fraction of the total energy output of the supernova.

Currently it is not possible to distinguish between the two proposed models for the prompt radio burst. If a major radio burst, such as has been observed in SN1979C and SN1980K, occurs in SN1987A, then the form of the rise in flux density at different frequencies should enable us to determine which model best describes the system. We are maintaining a regular monitoring of the radio emission from the supernova to detect such a rise at an early stage.

We thank J. L. Caswell and J. A. Roberts for discussions, the Director and staff of the NASA Deep Space Communications Complex for help with the observations and D. R. Brown and J. B. Corben for assistance with reduction of the Fleurs data. We particularly thank the staff of the Parkes radiotelescope for their assistance. The Molonglo and Fleurs telescopes are supported by the University of Sydney and the Australian Research Grants Scheme.

Received 30 March; accepted 10 April 1987.

- Shelton, I. *IAU Circ.* 4316 (1987).
- McNaught, R. H. *IAU Circ.* 4316 (1987).
- Shelton, I. *IAU Circ.* 4330 (1987).
- Phillips, M. *IAU Circ.* 4318 (1987).
- White, G. L. & Malin, D. F. *Nature* (in the press).
- Sonneborn, G. & Kirshner, R. *IAU Circ.* 4333 (1987).
- Mills, B. Y. *Proc. astr. Soc. Aust.* 4, 156-159 (1981).
- Mills, B. Y. & Turtle, A. J. in *Structure and Evolution of the Magellanic Clouds* (eds van den Bergh, S. & de Boer, K. S.) 283-291 (Reidel, Dordrecht, 1984).
- Bunton, J. D., Jones, I. G. & Brown, D. R. *Proc. astr. Soc. Aust.* 6, 93-100 (1985).
- Norris, R. P., Batty, M. J., Kesteven, M. J. & Wellington, K. J. *Proc. astr. Soc. Aust.* 6, 137-140 (1985).
- Weiler, K. W., Sramek, R. A., Panagia, N., van der Hulst, J. M. & Salvati, M. *Astrophys. J.* 301, 790-812 (1986).
- Haynes, R. F. *et al. Mon. Not. R. astr. Soc.* 185, 661-667 (1978).
- Chevalier, R. A. *Astrophys. J.* 259, 302-310 (1982).
- Gry, C., Cassatella, A., Wamsteker, W. & Sanz, L. *IAU Circ.* 4324 (1987).
- Crane, P. & Leventhal, M. *IAU Circ.* 4341 (1987).
- Rossi, B. & Greisen, K. *Rev. mod. Phys.* 13, 240-309 (1941).

Quasar spectra and the gravitational lens hypothesis

P. A. Shaver, E. J. Wampler & S. Cristiani

European Southern Observatory, Karl-Schwarzschild-Strasse 2,
D-8046 Garching bei München, FRG

Quasar spectra have been used to identify gravitational lens candidates¹⁻⁶, often in the absence of other evidence, and it has even been suggested⁷ that they can be used as reliably as fingerprints to uniquely identify individual objects. Here we point out that quasar spectra are sufficiently similar that, in the presence of noise, it can be extremely difficult to distinguish between different objects solely from spectral characteristics.

Quasars are found and identified on the basis of their being 'quasistellar' in appearance and having distinctive spectra. They form a class spectroscopically to such an extent that many attempts have been made to produce a 'composite quasar spectrum'⁸⁻¹⁰. Quasar spectra can certainly vary from object to object¹¹⁻¹⁴, but the transition from one extreme within the class to the other is continuous, so that two different quasars can have spectra which are virtually indistinguishable from each other, especially in the presence of noise.

Figure 1 shows typical high redshift spectra in the Lyman- α -C IV region (from ref. 15), which illustrate the 'family resemblance' among quasar spectra. Line widths and relative line strengths are distributed around characteristic values, and do not in themselves provide a unique signature. Furthermore, quasars with similar absolute luminosities have very similar line strengths^{12,14,16}. There is an anti-correlation between the equivalent width of the lines and the continuum luminosity; it has been suggested¹⁷ that the broad emission lines are produced in a matter-limited region and that this anti-correlation is caused

by the inability of the ionized region to expand as the continuum flux of the quasar increases. Even the presence of broad absorption lines is shared by 5-10% of all quasars. However, there can be considerable structure in these absorption lines, and considerable variation from one object to another, so they may serve better than emission lines for identification purposes. The narrow absorption lines are irrelevant, as they are thought to originate along the line of sight.

The Mg II $\lambda 2,800$ region of quasars has been extensively studied^{11,18-20}. The region is dominated by strong Fe II permitted lines. The relative strength of individual multiplets is approximately constant from object to object although differences can occur especially if fluorescence pumping is important²¹ or if the Doppler widths in the broad lines differ. Most quasars have strong Fe II line strengths, a fact that does not seem to have been appreciated. In fact, all quasars in the Bright Quasar Survey²² (BQS) that have been observed at Lick Observatory have very noticeable Fe II features (J. A. Baldwin and E.J.W., manuscript in preparation). When Fe II is strong, dips and bumps appear in the spectra, corresponding to the multiplet structure of Fe II, and it is not surprising that the spectra of different objects resemble each other. Other features, such as those at $\lambda 2,080$ and $\lambda 2,420$, may be due to Fe III permitted multiplets^{11,20} and might also be expected to have similar strengths in objects with similar ionization structure.

Figure 2 shows the Mg II $\lambda 2,800$ region in the spectra of eight quasars. Two of these are the wide-separation pair 1146+111 B and C, which were suggested to be gravitationally lensed images of a single object⁶ but on the basis of recent observations showing significant spectral differences at other wavelengths^{23,24} and negative results from searches for a massive lensing object are now thought more likely to be distinct quasars. Aside from the prominent Mg II emission line, features attributable primarily to the myriad Fe II multiplets dominate this spectral region and give it a characteristic pattern. The presence of this

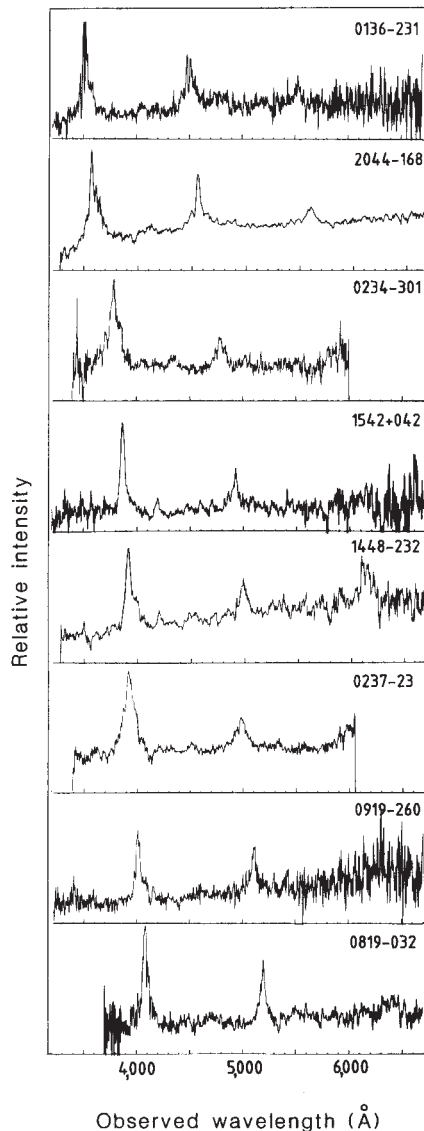


Fig. 1 Typical spectra of high-redshift quasars in the Lyman α -C-IV region, from ref. 15.

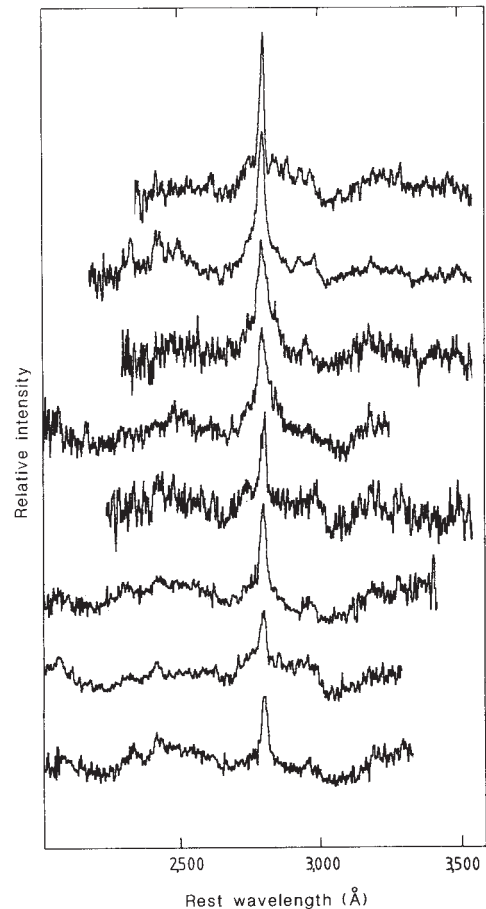


Fig. 2 Spectra of eight quasars in the vicinity of Mg II λ 2,800, reduced to the rest frame. For purposes of illustration the spectra have been straightened by fitting quadratic baselines, and they are all on the same scale relative to the continuum. Six of the eight are BQS quasars observed at Lick Observatory, and the other two are 1146+111B,C observed at La Silla; from top to bottom they are 1543+489, 2112+059, 1103-006, 0044+030, 1259+593, 1146+111B, 2302+029 and 1146+111C.

pattern does not uniquely identify an object, and the similarity between two spectra in this region is not particularly remarkable.

Because the overall appearance of quasar spectra is determined by relatively few parameters, only broad classification is possible. But unique identification requires far more than this, and quasar spectra of mediocre quality do not provide enough independent parameters. To make matters worse, quasar spectra can vary with time²⁵, so that two gravitationally lensed images of the same object, whose light paths differ significantly, may not be identical, even in redshift (for example, ref. 6, and F. Hoyle, preprint no. 88, Cardiff University (1983)).

What, then, are the prospects of using quasar spectra to distinguish cases of gravitational lensing from distinct quasars located in clusters of galaxies, or close pairs of quasars whose activity may have been mutually triggered by gravitational interactions? Figure 3 shows the discovery spectra for several 'established' gravitational lenses. In view of the above discussion, the similarity in spectral properties of the top four pairs is not unusual. The redshifts for each pair are certainly similar, although this would also be expected of distinct objects in clusters. The separation between the small vertical lines near the centre of each pair of spectra corresponds to $1,000 \text{ km s}^{-1}$ in the rest frame, and because the velocity dispersion of galaxies near low-redshift quasars is considerably less than this²⁶, it

would obviously be difficult to distinguish lensed cases on the basis of small redshift differences, even if the intrinsic spectra did not vary.

The bottom two pairs are unusual for quasars in that they have narrow emission lines, although such lines are common among many Seyfert galaxies, radio galaxies, and extragalactic H II regions (which often occur in pairs and can masquerade as gravitational lenses^{27,28}). These narrow emission lines (particularly the density-sensitive forbidden lines) probably originate in larger regions of lower density. The light travel-time across such regions is probably far greater than the difference in light travel-time along the two lines of sight, so that narrow lines appearing in the spectra of the two images should give the same redshift, and their intensity ratios relative to each other should be the same in both spectra. Such properties are necessary for the lens hypothesis, but not sufficient.

Perhaps the strongest evidence would come from the detection of identical (spectral) variations in both images, with an appropriate time lag because of the path difference along the two lines of sight. The absence of such a correlation could, however, conceivably be due to microlensing²⁹, and would therefore not in itself necessarily constitute an argument against lensing. Studies of polarization changes could probably establish the presence of a gravitational lens³⁰. Statistical arguments may also eventually be useful. The average redshift difference might perhaps be expected to be smaller, and the average spectra more similar, for the lensed images than for close physical pairs³¹,

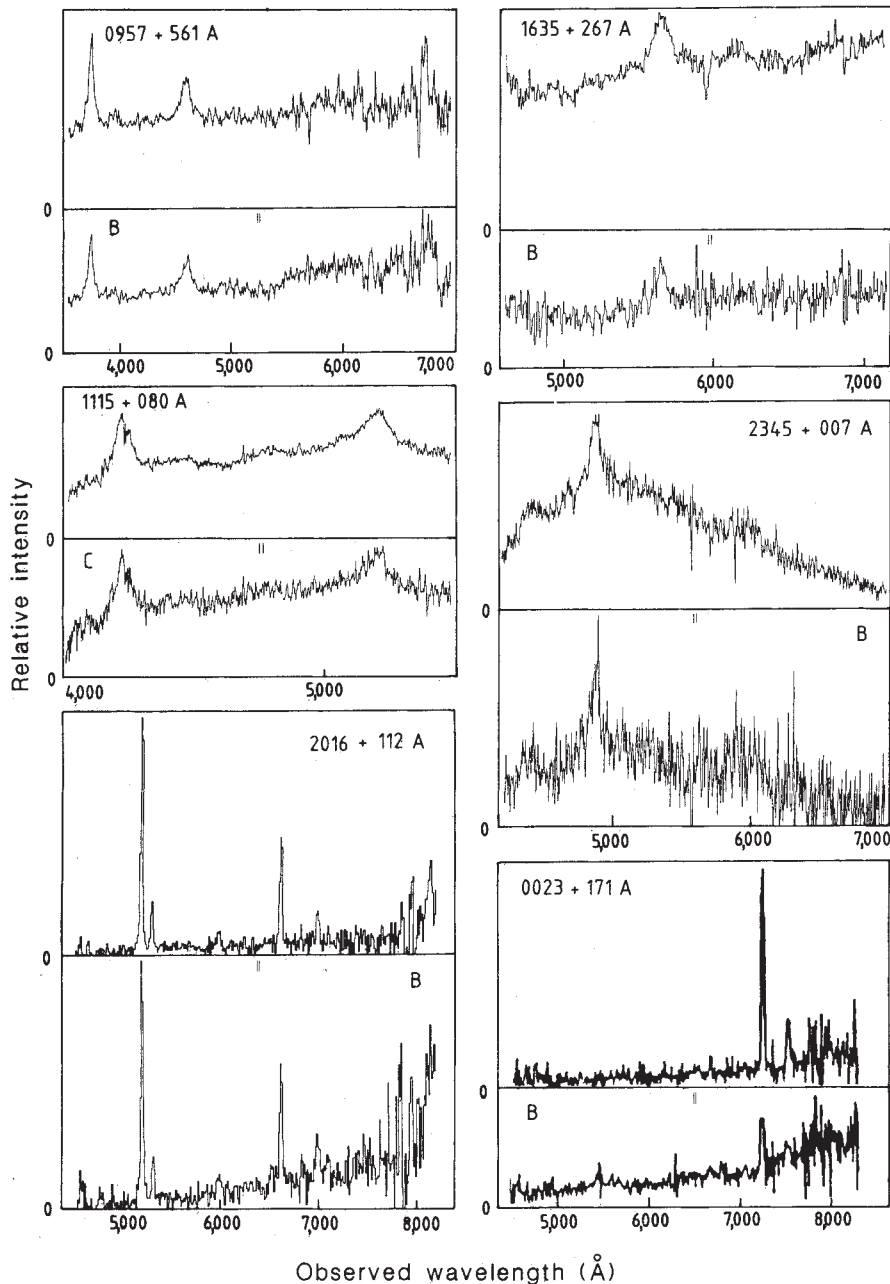


Fig. 3 Discovery spectra for six gravitational lens candidates, adapted from refs 1-5 and 33. The separation between the two small vertical lines near the middle of each pair of spectra corresponds to $1,000 \text{ km s}^{-1}$ in the rest frame.

although this is not obvious. Lensed quasars might be expected to have relatively high redshifts on average, as the probability of an intervening lensing mass is then greater, but the clustering of matter around quasars, hence the incidence of close quasar pairs, may also be greater at high redshift³², so again no clear distinction may be possible. Other possible pieces of evidence include the number of images in each case, the properties of any radio emission, the presence of a candidate lens, and the distribution in angular separation, particularly on sub-arc second scales. The case for gravitational lensing will have to be based on several such criteria—the spectroscopic data by themselves may not suffice.

Received 15 December 1986; accepted 27 January 1987.

- Walsh, D., Carswell, R. F. & Weymann, R. J. *Nature* **279**, 381-384 (1979).
- Weymann, R. J. *et al. Nature* **285**, 641-643 (1980).
- Weedman, D. W., Weymann, R. J., Green, R. F. & Heckman, T. M. *Astrophys. J.* **255**, L5-L9 (1982).
- Djorgovski, S. & Spinrad, H. *Astrophys. J.* **282**, L1-L4 (1984).
- Lawrence, C. R. *et al. Science* **223**, 46-49 (1984).
- Turner, E. L. *et al. Nature* **321**, 142-144 (1986).
- Waldrop, M. M. *Science* **232**, 936 (1986); *Time* May 19, 62 (1986).
- Osterbrock, D. E. & Parker, R. A. R. *Astrophys. J.* **143**, 268-270 (1966).
- Davidson, K. *Astrophys. J.* **171**, 213-231 (1972).
- Chan, Y.-W.T. & Burbidge, E. M. *Astrophys. J.* **198**, 45-55 (1975).
- Hartig, G. F. & Baldwin, J. A. *Astrophys. J.* **302**, 64-80 (1986).
- Wampler, E. J., Gaskell, C. M., Burke, W. L. & Baldwin, J. A. *Astrophys. J.* **276**, 403-412 (1984).
- Wilkes, B. J. *Mon. Not. R. astr. Soc.* **218**, 331-361 (1986).
- Kinney, A. L., Huggins, P. J., Gassgold, A. E. & Bregman, J. N. *Astrophys. J.* (in the press).
- Wilkes, B. J., Wright, A. E., Jauncey, D. L. & Peterson, B. A. *Proc. astr. Soc. Aust.* **5**, 2 (1983).
- Baldwin, J. A. *Astrophys. J.* **214**, 679-684 (1977).
- Wamsteker, W. & Colina, L. *Astrophys. J.* **311**, 617-622 (1986).
- Grandi, S. A. *Astrophys. J.* **255**, 25-38 (1982).
- Wills, B. J., Netzer, H. & Wills, D. *Astrophys. J.* **288**, 94-116 (1985).
- Wampler, E. J. *Astr. Astrophys.* **161**, 223-231 (1986).
- Netzer, H. & Wills, B. J. *Astrophys. J.* **275**, 445-460 (1983).
- Schmidt, M. & Green, R. F. *Astrophys. J.* **269**, 352-374 (1983).
- Shaver, P. A. & Cristiani, S. *Nature* **321**, 585-586 (1986).
- Huchra, J. P. *Nature* **323**, 784-786 (1986).
- Gaskell, C. M. & Sparke, L. S. *Astrophys. J.* **305**, 175-186 (1986).
- Heckman, T. M., Bothun, G. D., Balick, B. & Smith, E. P. *Astr. J.* **89**, 958-965 (1984).
- Chen, J.-S. & Shaver, P. A. *The Messenger* No. **30**, 2 (1982).
- Shaver, P. A. & Chen, J.-S. *Astr. Astrophys.* **148**, 443-446 (1985).
- Kayser, R., Refsdal, S., Stabell, R. *Astr. Astrophys.* **166**, 36-52 (1986).
- Keel, W. C. *Astrophys. J.* **255**, 20-24 (1982).
- Turner, E. L. in *Observational Cosmology* (IAU Symp. No. 124, eds Burbidge, G. & Hewitt, A.) (in the press).
- Yee, H. K. C. & Green, R. F. in *Quasars* (IAU Symp. No. 119, eds Swarup, G. & Kapahi, V. K.) 481-487 (Reidel, Dordrecht, 1986).
- Burke, B. F. in *Quasars* (IAU Symp. No. 119, eds Swarup, G. & Kapahi, V. K.) 517-527 (Reidel, Dordrecht, 1986).

VHF radar observations of cat's-eye-like structures at mesospheric heights

Iain M. Reid, Rüdiger Rüster & Gerhard Schmidt

Max-Planck-Institut für Aeronomie, D-3411 Katlenburg-Lindau, FRG

Observations of noctilucent clouds indicate the frequent occurrence of wave-like motions and Kelvin-Helmholtz billows at the high latitude summer mesopause^{1,2} (~83 km), but to date no other technique appears to have produced direct evidence of their presence at these heights³. High-powered mesosphere-stratosphere-troposphere very high frequency Doppler radars with good height and time resolution (~150–300 m and ~10 s respectively) capable of making such observations have recently become available, and the backscattering regions in the mesosphere detected by such radars often take the form of thin layers that appear to be produced by dynamical instabilities in the region of maximum wind shear⁴. Such wind conditions should produce Kelvin-Helmholtz instabilities, and here we present two examples of mesospheric radar observations that suggest the characteristic 'cat's-eye'-like turbulent structures often associated with them in other parts of the atmosphere⁵, the ocean⁶ and in laboratory experiments⁷.

The mesosphere-stratosphere-troposphere radar technique has been described in a number of publications^{8,9}. It uses irregularities in refractive index at half the radar wavelength ($\lambda/2 \sim 3$ m in our case) to provide backscatter. These irregularities are assumed to move with the background wind, and by measuring their Doppler shifts in a minimum of three directions, the three wind components may be obtained. The Sousy 50 MHz very high frequency (VHF) radars that were used to obtain the results presented here are described in Czechowsky *et al.*¹⁰. They are located in the Harz Mountains of Germany (51° N, 16° E), and on the island of Andoya in Norway (69° N, 16° E). The only differences between them relevant to the current work are related to the radar beam arrangements, and to the beam-sampling sequences used.

The radar on Andoya was configured so that beams were directed vertically (V), at 4° from the zenith towards the north-west (NW), and at 4° from the zenith towards the north-east (NE). Its beamwidth was 3° and it was directed for 11 s in each of the different beam directions in the following sequence: NE, NW, V. The radar in the Harz Mountains was arranged so that beams were directed at 7° from the zenith towards the north (N), south (S), east (E) and west (W), and also vertically. The beamwidth of this radar is 5°, and it was directed for 11 s in each beam direction in the following sequence: E, W, E, W, E, W, N, S, N, S, N, S, V, V, V, V, V. The power, mean Doppler shift and the width of the Doppler spectrum of each 11-s sample of backscattered radio waves were calculated in the usual manner for these types of observations¹¹. We sampled 120 height gates of 300 m beginning at 49.8 km on Andoya, and at 60 km in the Harz.

Figure 1a, b, is a height-time intensity plot of the power of the backscattered radio waves measured in the vertical beam using the Harz radar on 12 November 1985. Each pixel represents spatial and temporal dimensions of 300 m and 11 s respectively. The darker pixels correspond to higher power levels, and consequently to higher levels of 3-m scale turbulence. The intensity or power range between the blank and darkest screen points represents the range in power in seven steps, for that 11 s, for the height range shown. The intermediate grey shades vary by one-seventh of the range in power in decibels. Also, as a consequence of the sampling sequence used, the timescale is intermittent, representing 1.1 min every 3.3 min.

Inspection of this diagram indicates a well-defined layer between 69 and 72 km, within which additional structure is

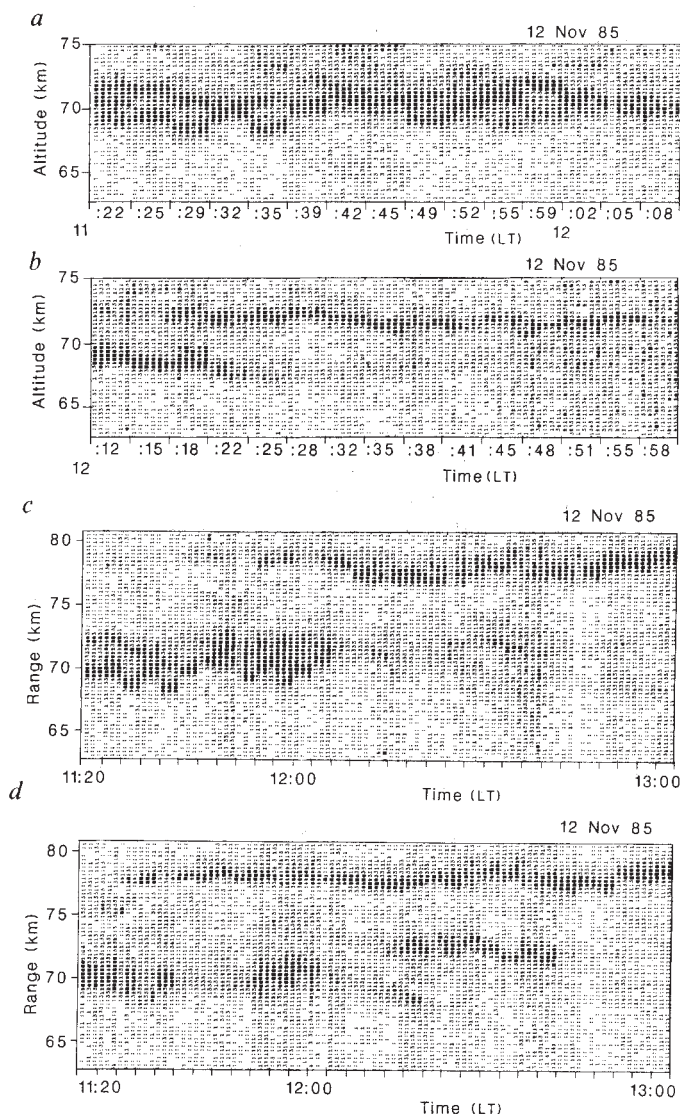


Fig. 1 a, Height-time intensity plot of the power of the backscattered radio waves measured in the vertically directed beam of the Harz radar on 12 November 1985. Pixels are 300 m $\times$ 11 s. Darker pixels represent higher power levels, and hence more intense 3-m turbulence. The radar was directed vertically for 1.1 min in 3.3 min. The times shown refer to the beginning of the associated interval and are rounded to the nearest minute. b, a continued. c, As for a and b but for the westward radar beam. d, As for a and b but for the southward radar beam.

evident. The broken timescale gives a slightly misleading image of this structure, but careful inspection reveals the presence of two thin layers with a separation of about 2 km at 11:29 and 11:35 LT, but only one thin layer at 11:32 LT. Other similar variations in structure are easily located. After 11:59 LT, the layer exhibits none of these cat's-eye like features, and at 12:18 LT two layers separated by about 4 km become clearly evident. A similar kind of evolution, from cat's-eyes to twin layers, has been suggested by a number of studies as being typical for Kelvin-Helmholtz instabilities¹². A similar braided structure is evident in the westward beam (Fig. 1c), but not in the southward beam (Fig. 1d), but both suggest the development of twin layers.

Figure 2a, b shows the mean westward and northward radial velocities corresponding to Fig. 1a (11:20–12:16 LT). In Fig. 2a the radial velocity is positive eastward. The presence of wave-like features is clearly suggested in both components. These examples were chosen from the four available off-zenith beams because they have the best height coverage. One limitation of

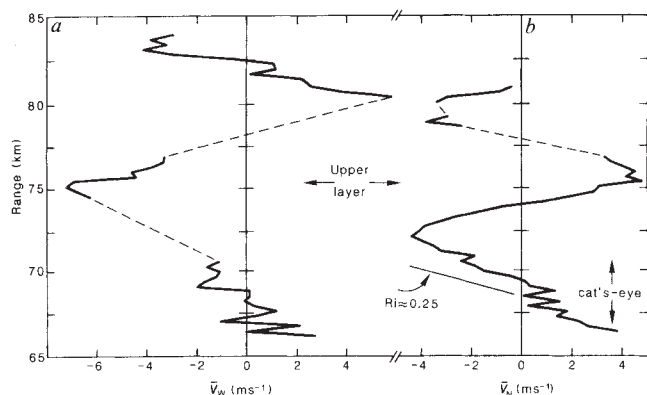


Fig. 2 *a*, Mean westward ($\bar{V}_W$) radial velocity for 11:20–12:16 LT for 12 November 1985. This interval corresponds to Fig. 1*a*. Values have been plotted so that they are positive eastward. To convert radial velocities to horizontal velocities using the assumption of zero vertical velocity, these values should be multiplied by 9.1. *b*, Mean northward ($\bar{V}_N$) radial velocity for 11:20–12:16 LT. The shear required to produce a Richardson number of 0.25 for buoyancy frequency of 0.02 s^{-1} is indicated by a straight line.

the VHF-MST technique for mesospheric work is that it requires both sufficient turbulence and free electrons to obtain backscatter and hence radial velocities. Inspection of Fig. 1 clearly shows the temporally and spatially intermittent nature of the backscattering regions. Better height coverage can be obtained by incoherently adding individual Doppler spectra, but the time resolution is thereby restricted.

Calculation of the vertical wavelength from the height variation of the mean horizontal wind component for the entire observational period yields a value of 7.5 km, and this is consistent with the radial velocities shown in Fig. 2. The profiles for the second half of the observations show negligible phase progression in relation to those shown in Fig. 2. The upper layer evident in Fig. 1*c, d* also shows little evidence of upward or downward propagation. The components are also almost in phase quadrature, and have similar perturbation amplitudes. These facts suggest the height variations in the wind profiles is due to a long period (inertio-) gravity wave or to a tidal period motion. Unfortunately the data length (1 h 54 min) is too short to obtain an even moderately accurate measure of the period. The range of occurrence of the braided structure shown in Fig. 1*a* is indicated in Fig. 2. In the height interval in which this structure occurs, the shear clearly varies, from values in the periphery that correspond to a Richardson number, $Ri \sim 0.25$ if a typical buoyancy frequency of 0.02 s^{-1} is assumed, to lower values in the central region. This is consistent with an initial

shear that is large enough to produce instability being reduced as the instability develops.

The upper layer shown in Fig. 1*c, d* was evident in all five beam directions. It exhibits a very clear variation in range, which in the northward, southward, westward and vertical beam directions have a similar period, about 20–30 minutes, with a vertical variation (peak to peak) of between 0.9 and 2 km. However, in the eastward beam it shows little evidence of periodicity. Superimposed on this long period oscillation are shorter period (~ 5 – 10 min) fluctuations. A simple cross-correlation analysis of the maximum-power height, for the northward, southward and vertical beams of the time series indicates that the north-south undulation in power has a speed of about 18 ms^{-1} . This agrees well with the background meridional wind speed of 19 ms^{-1} at the mean height of occurrence of the upper layer. Although the height profiles shown in Fig. 2 are incomplete, it seems clear the upper layer is also associated with large shears in both eastward and northward wind components, and that the magnitude of the shear is comparable with that indicated for $Ri \approx 0.25$. The close agreement between the speed of the undulation in power and the wind velocity (at least for the meridional component) at the same height, and the presence of oscillations with frequencies near the buoyancy frequency make it possible to suggest that this layer has also been produced by some kind of dynamical instability. Application of the approximate dispersion relation¹³ $|c - \bar{u}| = N/m$, where c is the horizontal phase velocity, $\bar{u}$ the mean wind in the direction of wave propagation, and m the vertical wavenumber, yields an intrinsic phase velocity $|c - \bar{u}|$ of $\sim 25 \text{ ms}^{-1}$ for a vertical wavelength of 7.5 km. This is rather close to the peak horizontal perturbation velocity calculated at the mean height of the upper layer ($\sim 26 \text{ ms}^{-1}$), and because the condition for convective (or Rayleigh–Taylor) instability is that the perturbation velocity equal or exceed the intrinsic phase velocity¹⁴, it is possible that the upper layer has been produced by convective instability. However it is not possible to confirm this in the present case, and KH instability would be an equally likely candidate.

Re-analysis of data previously interpreted as providing indirect evidence of KH instability¹⁵ produces a rather less ambiguous example of the familiar cat's-eye-like form when presented as height-time intensity plots (Fig. 3). This diagram shows data obtained using the radar on Andoya on 1 February 1984 in a three beam mode. Each pixel represents $300 \text{ m} \times 11 \text{ s}$, with a 33-s interval between adjacent pixels. In the vertical beam the turbulent structure at 77 km shows the development from partial to mature cat's-eye like structures. The 'overlapping' turbulent layers evident at 11:20 LT and 11:35 LT are typical of many radar observations of KH instability in the troposphere¹⁶. Similar features are present in the other beams, but there are variations due to the differing beam orientations. After the

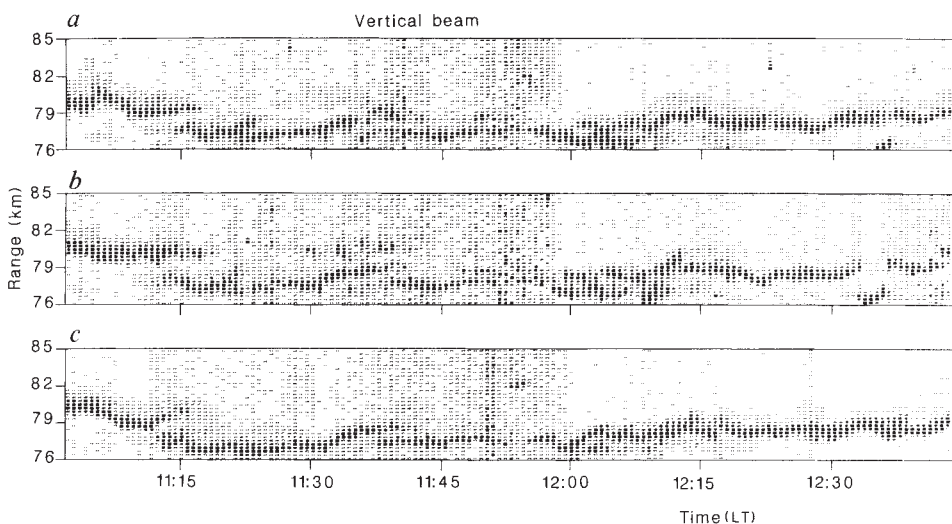


Fig. 3 Height-time intensity plot of the power of the backscattered radio waves measured in *a*, vertical; *b*, north-west; and *c*, north-east beams of the Andoya radar on 1 February 1984. Pixels are $300 \text{ m} \times 11 \text{ s}$, and are separated by 33 s.

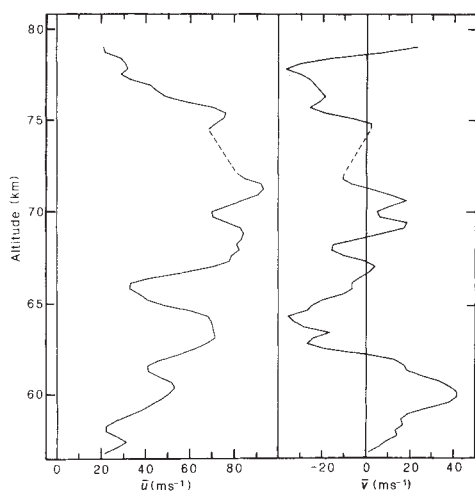


Fig. 4 Mean westward ($\bar{u}$) and northward ($\bar{v}$) horizontal velocities for 11:01–12:18 LT for 1 February 1984.

mature cat's-eye feature disappears, the layer continues to oscillate with a period near the Vaisala–Brunt period. The horizontal wind components for 11:01–12:18 LT are shown in Fig. 4. The structures shown in Fig. 3 are clearly associated with a large shear in the eastward component. These profiles also show evidence of shorter vertical wavelength waves (~ 4 km), that appear to be associated with downward propagating turbulent layers¹⁵. The 77-km layer shows little evidence of upward or downward propagation and would appear to be associated with a longer period gravity wave or tidal period motion.

The cat's-eye like structure in Fig. 3 is similar to that seen in Fig. 1a, with a vertical extent of about 2 km, and an interval of about 5 min between the centres of the cat's-eyes. This feature propagated at the background wind speed¹⁵ (~ 30 m s⁻¹) suggesting a horizontal extent of about 9 km, so that the vertical to horizontal aspect ratio is consistent with the 1 to 7 suggested as being typical for KH instabilities³. A value of 9 km is also in agreement with estimates of horizontal extent based on noctilucent cloud observations¹⁷, and with rocket trail observations at heights near 107 km (ref. 18). While the possibility exists that these variations represent the intermittent formation of two thin layers by some other mechanism, the similarity of the turbulent structures evident in Fig. 1a and Fig. 3 suggests that the former are associated with KH instability.

The observations presented in Fig. 1 are clearly not ideally suited to investigations small-scale structure and are too limited in time to allow an estimate of the period of the long-period gravity wave that appears to be subject to the dynamical instability to be obtained. Further observations that may allow more detailed investigation are being conducted. The February observations represent the best example of braided mesospheric structures to which we have access. The fact that they are rather noisier and less clear than typical tropospheric observations is due to the rather more complex wavefield at mesospheric heights, and to the fact that the radar is operating near its limits. Indeed, the results shown in Figs 1 and 3 appear to be the first radar observations of cat's-eye-like structures at mesospheric heights.

Received 7 November 1986; accepted 24 February 1987.

1. Witt, G. *Tellus* **14**, 1–12 (1962).
2. Haurwitz, B. & Fogle, B. *Deep Sea Res.* **16**, 85–95 (1969).
3. Fritts, D. C. & Rastogi, P. K. *Radio Sci.* **20**, 1247–1277 (1985).
4. Rüster, R. *Adv. Space Res.* **4**, 3–18 (1984).
5. Röttger, J. & Schmidt, G. *IEEE Trans. Geosci. Electron.* **17**, 182–189 (1979).
6. Woods, J. D. *J. Fluid Mech.* **32**, 791–800 (1968).
7. Thorpe, S. A. *J. Fluid Mech.* **85**, 7–31 (1978).
8. Balsley, B. B. & Gage, K. S. *Pure appl. Geophys.* **118**, 452–493 (1980).

9. Harper, R. M. & Gordon, W. E. *Radio Sci.* **15**, 195–211 (1980).
10. Czechowsky, P. et al. *Radio Sci.* **19**, 441–450 (1984).
11. Woodman, R. F. *Radio Sci.* **20**, 1185–1195 (1985).
12. Browning, K. A. & Watkins, C. D. *Nature* **227**, 260–263 (1970).
13. Fritts, D. C. *Rev. Geophys.* **22**, 275–308 (1984).
14. Orlanski, I. & Bryan, K. *J. geophys. Res.* **71**, 6975–6983 (1969).
15. Rüster, R. & Klostermeyer, J. *J. atmos. terr. Phys.* (in the press).
16. Browning, K. A. *Q. Jl R. met. Soc.* **97**, 283–299 (1971).
17. Fogle, B. & Haurwitz, B. *Space Sci. Rev.* **6**, 279–340 (1966).
18. Lloyd, K. H. et al. *Plan. Space Sci.* **21**, 653–661 (1973).

Vibrational anomalies in the superconducting compound $\text{La}_{1.85}\text{Ba}_{0.15}\text{CuO}_4$

G. Balakrishnan*, N. R. Bernhoeft†, Z. A. Bowden‡, D. McK Paul* & A. D. Taylor‡

* Department of Physics, University of Warwick, Coventry CV4 7AL, UK

† Department of Physics, University of Durham, Durham DH1 3LE, UK

‡ Rutherford Appleton Laboratory, Chilton, Oxfordshire, OX11 0QX, UK

$\text{La}_{1.85}\text{Ba}_{0.15}\text{CuO}_4$ is the prototype member of a new class of unusual high temperature ($T_c > 24$ K) superconductors. These novel materials whose properties lie at the interface between metallic and insulating behaviour show a wide variety of physical phenomena. For example, the electrical resistivity of the parent compound La_2CuO_4 shows metallic behaviour down to approximately 25 K where an abrupt upturn occurs towards a semiconducting or insulating state. The barium-doped superconductor exhibits a linear fall in resistivity from room to liquid-nitrogen temperatures followed by a plateau to 35 K, at which point superconducting behaviour begins. Several theoretical suggestions regarding the origin of this behaviour have been put forward, but the role played by dynamic or static lattice instabilities is still an area of controversy (see refs 1–4). Evidence for the presence of a tetragonal to orthorhombic phase transition together with further subtle and anomalous structural instabilities in the barium-doped compound has been reported⁵. To elucidate the correlations between the lattice vibrational and electronic behaviour, we have undertaken neutron inelastic scattering experiments on the High Energy Transfer spectrometer at the ISIS facility of the Rutherford Appleton Laboratory, UK, using carefully prepared polycrystalline samples of both $\text{La}_{1.85}\text{Ba}_{0.15}\text{CuO}_4$ and La_2CuO_4 . The experiments were performed over a range of temperatures between 12 K and 200 K. Here we report anomalous temperature dependence of modes centred at 10 meV and 20 meV respectively.

The materials used in these experiments were prepared by a careful and thorough mixing of solutions of high-purity reagents ($\text{La}(\text{NO}_3)_3$, $\text{Cu}(\text{NO}_3)_2$ and $\text{Ba}(\text{NO}_3)_2$) in appropriate cation proportion followed by precipitation with a standard solution of sodium carbonate. The precipitate was carefully washed, dried and fired at a temperature of 1,100 K for 2 hours. This material was then ground, processed into pellets and sintered for a further 4 hours at 1,400 K. Samples made in this way showed a sharp superconducting transition at 35 K with no low-temperature tail.

To study the inelastic response over the temperature range 10 K–200 K and for energy transfers up to 100 meV an incident neutron energy of 130 meV was used and data taken in neutron energy loss. Data were collected in three detector banks situated at mean scattering angles (angular width) of 5 (3.6), 20 (20) and 123 (1) degrees respectively. From the spectra obtained we have been able to estimate the relative weight of multi-phonon (single events in which the neutron exchanges more than one phonon with the lattice) and multiple phonon (neutron scattered more than once, with exchange of phonons, before emerging from the crystal) scattering; the contribution of such events was small and unstructured over the temperature and energy ranges

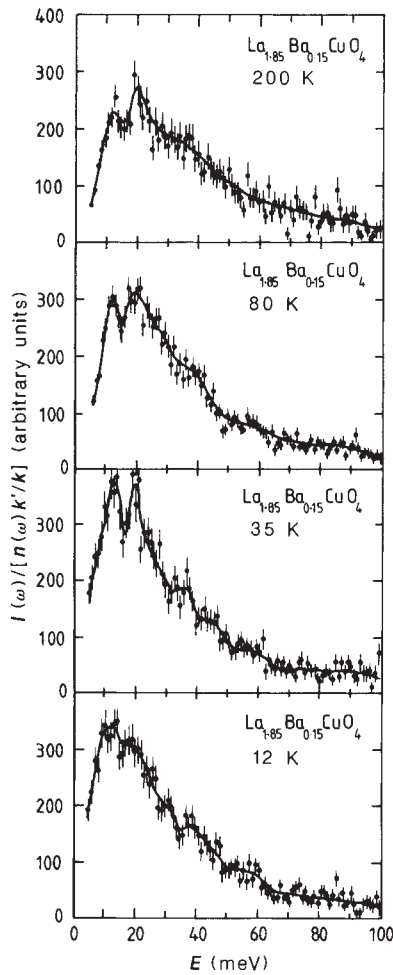


Fig. 1 The observed neutron energy loss inelastic scattering intensity from the high temperature superconductor ($T_c = 35$ K) $\text{La}_{1.85}\text{Ba}_{0.15}\text{CuO}_4$ corrected for background, detector efficiency, the ratio of final to incident wavevector and population factor. The chosen temperatures of 200 K, 80 K, 35 K and 13 K are representative of important temperature regimes in view of the observed structural anomalies in this compound. The solid line serves as a guide to the eye.

of interest. In contrast, despite using thin samples (91% transmission), it is prohibitively difficult to make accurate corrections for Bragg plus one phonon (indirect) scattering events in our materials. Such double scattering events are clearly discernible in the data taken at small scattering angles where they provide a dominant contribution. A similar contribution is anticipated in the back-scattering detector bank but here, on account of the rapid increase of the one phonon (direct) cross-section which rises approximately as the square of the wavevector transfer, the contamination due to such processes is negligible. In any event the temperature dependence of the energy distribution profile of both direct and indirect scattering events is principally governed by a single Bose population factor, and hence the small contamination at high angles due to indirect processes will not detract from our conclusions concerning the anomalous temperature dependence of the observed scattering.

The partial differential cross-section for nuclear scattering may be written in the following style⁶

$$\frac{d^2\sigma}{d\Omega dE'} = \frac{1}{2\pi\hbar} \frac{k'}{k} \sum_{jj'} b_j b_{j'} \int_{-\infty}^{\infty} dt \exp(i\omega t) \times \langle \exp(-i\mathbf{Q} \cdot \mathbf{R}_{j'}) \exp(i\mathbf{Q} \cdot \mathbf{R}_j(t)) \rangle$$

where k' and k are the magnitude of the scattered and incident wavevectors respectively, b_j is the nuclear scattering length of atom j with position $\mathbf{R}_j$. $\mathbf{Q}$ is the difference $\mathbf{k}' - \mathbf{k}$ and the

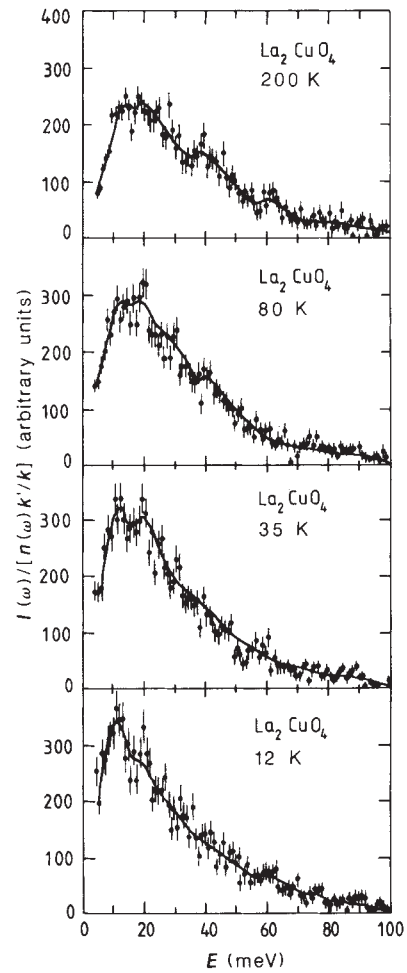


Fig. 2 The observed neutron scattering intensity from the reference compound La_2CuO_4 corrected as described in the caption to Fig. 1. The solid line serves as a guide to the eye.

remaining symbols have their usual meaning. The sum over scattering lengths gives rise to two terms, one of a 'self' or 'incoherent' form where $j = j'$ which gives rise to no spatial interference effects, and the other where $j \neq j'$ a coherent term which in the presence of an ordered lattice yields scattering that is highly structured in reciprocal space. In performing a polycrystalline sum at high incident energy, sufficient averaging over the Brillouin zone is achieved such that this scattering has an energy profile similar to that of the self term⁷⁻⁹.

In a one phonon approximation, and neglecting small resolution corrections (estimated to be $\sim 1\%$ at 100 meV), the observed scattered intensity takes the form,

$$I(\omega) = \text{constant} \frac{k'}{k} n(\omega) \sum_{d,s} \frac{b_d^2}{M_d} \frac{1}{\omega_s} F_{d,s}(\omega) \delta(\omega - \omega_s)$$

where $n(\omega)$ is the population factor, the sum d is over the atoms in the unit cell and the sum s is over the eigenfrequencies, M_d and b_d are the mass and the nuclear scattering length for atom d respectively. The factors $F_{d,s}(\omega)$, which contain terms like the Debye-Waller factor, are unknown and depend in detail on the

Table 1 Neutron cross-section for inelastic scattering

Element	b_d^2/M_d (mbarns/a.m.u.)
La	4.96
Ba	1.97
Cu	9.08
O	21.0

eigenvectors of the phonon modes of the crystal. But under the given experimental conditions, it is a reasonable assumption that these factors are weakly frequency- and temperature-dependent. The high-angle bank data, presented in Figs 1 and 2 for $\text{La}_{1.85}\text{Ba}_{0.15}\text{CuO}_4$ and La_2CuO_4 respectively, have had both prefactors k'/k and $n(\omega)$ removed after the subtraction of empty cryostat scattering and a scaling for detector efficiency.

The key features are already present in the data at 200 K (top panels in Figs 1 and 2) where an enhancement of peaks centred at 10 meV and 20 meV is seen in the superconducting compound. This enhancement grows with decreasing temperature reaching a maximum at 35 K, coincident with the onset of superconductivity. This is followed by a collapse of scattering intensity, principally in the higher energy peak, in the low temperature phase (lower panel Fig. 1).

It is of interest to note a similar but less pronounced progression in the parent compound. In particular, an anomalous increase in intensity at 35 K precedes a low-temperature collapse which, in this instance, is accompanied by an electronic instability towards a semi-conducting or insulating state.

These experiments suggest that in both materials the behaviour of the conduction electrons and vibrational degrees of freedom of the lattice are strongly coupled. The neutron cross-section for inelastic nuclear scattering is more sensitive to oxygen than the other elements present (Table 1) and earlier high-resolution neutron diffraction data⁵ indicated anomalous motions of the oxygen atoms. On this basis, it is plausible to associate the unusual behaviour of the measured vibrational spectra with motions involving the oxygen atoms. A further important point concerns the evaluation of heat capacity data (these measurements are being carried out); here the crucially important electronic contribution at the superconducting phase transition may only be estimated if the lattice part is known.

Received 2 April; accepted 14 April 1987.

1. Anderson, P. W. *Science* **235**, 1196 (1987).
2. Prelovsek, P., Rice, T. M. & Zhang, F. C. *J. Phys. C Lett.* (in the press).
3. Jorgensen, J. D. *et al. Phys. Rev. Lett.* **58**, 1024 (1987).
4. Mattheiss, L. F. *Phys. Rev. Lett.* **58**, 1028 (1987).
5. Paul, D. McK., Balakrishnan, G., Bernhoeft, N. R., David, W. I. F. & Harrison, W. T. A. *Phys. Rev. Lett.* (Submitted).
6. Marshall, W. & Lovesey, S. W. *Theory of Thermal Neutron Scattering: The Use of Neutrons for the Investigation of Condensed Matter* (Oxford University Press, 1971).
7. Bredov, M. M., Kotov, B. A., Okuneva, N. M., Oskotskii, V. S. & Shakh-Budogov, A. L. *Soviet Phys. & Solid St.* **9**, 214 (1967).
8. Kothari, L. S. *Soviet Phys. JETP* **20**, 1422 (1964).
9. Schweiss, P., Renker, B., Schneider, E. & Reichardt, W. *Proc. Second Rochester Conf. on Superconductivity in d and f band Metals* (ed. Douglass, D. H.) (Plenum, New York, 1976).

Small-scale structures in the north-west Atlantic sub-tropical front

John M. Toole & Raymond W. Schmitt

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

Mixing in the ocean thermocline is thought to occur in part by random superposition of internal waves, leading to occasional episodes of shear instability¹. Statistical models of the resulting mixing have been formulated^{2,3} which are roughly consistent with the few available direct estimates of average mixing rates⁴⁻⁶. However, clear evidence of the direct association between individual mixing patches and wave instability has been lacking⁷. Here we report the use of a new fine- and microstructure profiler during the Frontal Air-Sea Interaction Experiment (FASINEX)⁸ to investigate mixing processes in the sub-tropical front of the north-west Atlantic in great detail. The novel combination of fine-scale and microstructure sensors on one vehicle provided sufficient resolution to reveal quantitative evidence of shear instability in the thermocline. The strongest sub-surface mixing was found in associ-

ation with small-vertical-scale internal waves that may have been influenced by the velocity and density structure of the front.

The new instrument is a free-fall profiler very similar in design to the Total Ocean Profiler (TOPS) of S. Hayes of the Pacific Marine Environmental Laboratory⁹. Data from acoustic current meters, accelerometers and magnetometers are used in a model of the profiler's dynamic response to generate high resolution profiles of horizontal velocity. Temperature, conductivity and pressure are sensed with a Neil Brown conductivity-temperature-depth (CTD) instrument, temperature and conductivity microstructure with probes from Sea Bird Electronics and velocity microstructure with airfoil shear probes¹⁰ provided by Neil Oakey of the Bedford Institute of Oceanography.

Deployments of the instrument were made from the RV *Endeavor* during February and March 1986 in the region 27–29° N and 67–71° W. Here we report on the data obtained in a 24-h period spanning March 5–6 1986, three days after an intense storm passed through the area. This storm may have played a role in exciting the fine-scale structures we observed. The FASINEX front at this time was characterized by a two-layer temperature (and density) profile within 10–20 km south of the surface expression of the front¹¹. High resolution temperature profile data obtained with a wire-lowered CTD instrument allowed a detailed view of the cross-front extent of this structure (Fig. 1). Surface mixed-layer temperatures increased from 21.0 °C to 22.6 °C across the front; the maximum surface temperature gradient was 0.5 °C per 1.5 km. Deeper in the water column a 21 °C (temperature between 20.9 and 21.2 °C) thermostat was located at depths of 100–200 m. The thermostat, which appears to extend a distance of 15 km along the section, was composed of waters indistinguishable in temperature-salinity characteristics from the surface mixed-layer water on the cold side of the front. It has been suggested that the thermostat waters had recently been subducted at the FASINEX front¹¹.

Three deployments of the fine- and microstructure profiler were made within a 6-h period before the CTD section work. In terms of the hydrographic structure of the front, these deployments were located about CTD lowering number 11 as depicted in Fig. 1. The temperature and salinity profiles at this site also revealed the highly layered vertical structure of mixed layer and 21 °C thermostat (Fig. 2). The horizontal velocity profiles obtained with the fine- and microstructure profiler at this site are most remarkable. As was the case for temperature and salinity, the velocity appears layered in the vertical. A velocity jump, most prominent in the meridional component of this profiler drop, was found at 70–100 m: the boundary between the mixed layer and the 21 °C thermostat. Similarly, at the base of the thermostat, a second velocity jump occurred, most evident in this case in the zonal component. The observations during the 6-h period documented a clockwise rotation of the thermostat velocity vector with time. The sense and rate of this rotation is consistent with the thermostat undergoing an inertial oscillation in addition to advection by the velocity field of the front.

Superimposed on the step-like velocity profiles of the warm water side of the FASINEX front was an energetic fine-scale velocity field as is seen in Fig. 2. An expanded view of the velocity structure from one of the dives (Fig. 3) reveals short, vertical wavelength internal waves in which the velocity vector appears to rotate anticlockwise with depth, a characteristic which can be interpreted as upward vertical energy propagation¹². Similar structure was seen on the dive preceding that of Fig. 3; the following dive samples a packet characterized by clockwise rotation with depth at this level.

The depth intervals occupied by these waves were also distinguished by relatively intense patches of temperature and velocity microstructure (Fig. 3). These perturbations, with vertical scales of order 1 cm, are associated with ocean turbulence¹³. A candidate mechanism responsible for the turbulence is shear instability of the fine-scale internal wave velocity field. To explore this possibility, Richardson number profiles were estimated ($Ri =$

Fig. 1 Temperature profiles from the CTD obtained along a section across the FASINEX front. The temperature axis is correct for the first profile, taken near the surface expression of the front. Successive profiles are offset to the right by 1.0°C. These data were obtained by rapidly cycling the CTD over the depth interval 30–400 m while the ship slowly manoeuvred across the front into warmer water. Each profile was separated in space by roughly 1 km and 20 minutes in time. Fine- and microstructure profiler deployments were made near lowering number 11.

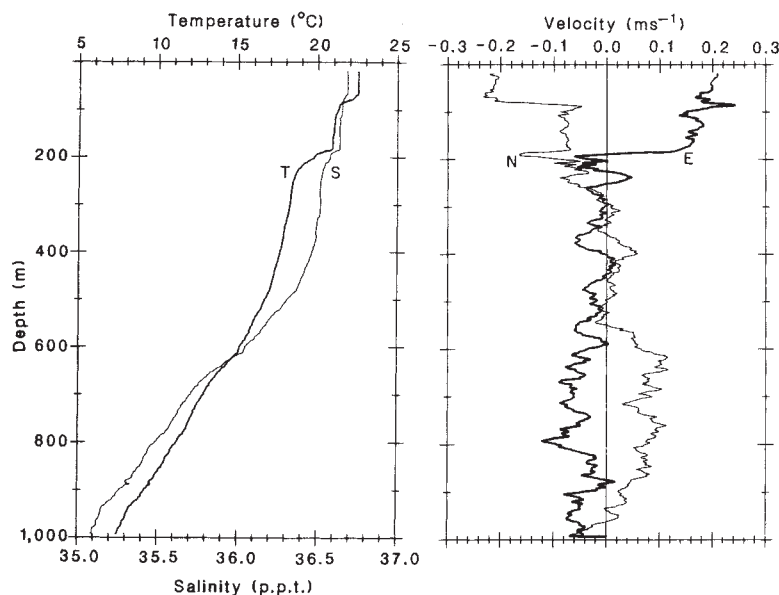
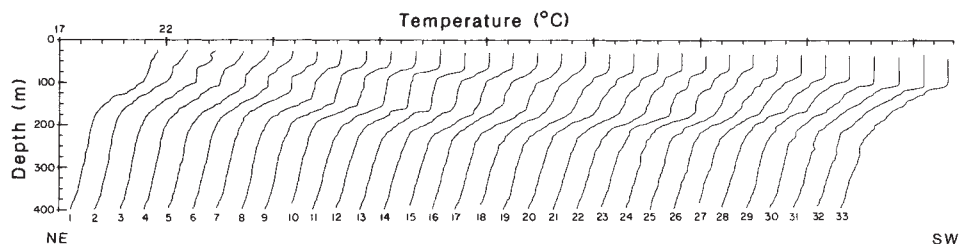


Fig. 2 Profiles of temperature, salinity and horizontal velocity obtained from deployment number 34 of the fine- and microstructure profiler. The velocity profile, which is relative to the average flow in the depth interval 20–1,000 m, was constructed using a modified version of a point-mass model applicable to free-fall ocean profilers⁹. The zonal component is indicated by the thick line.

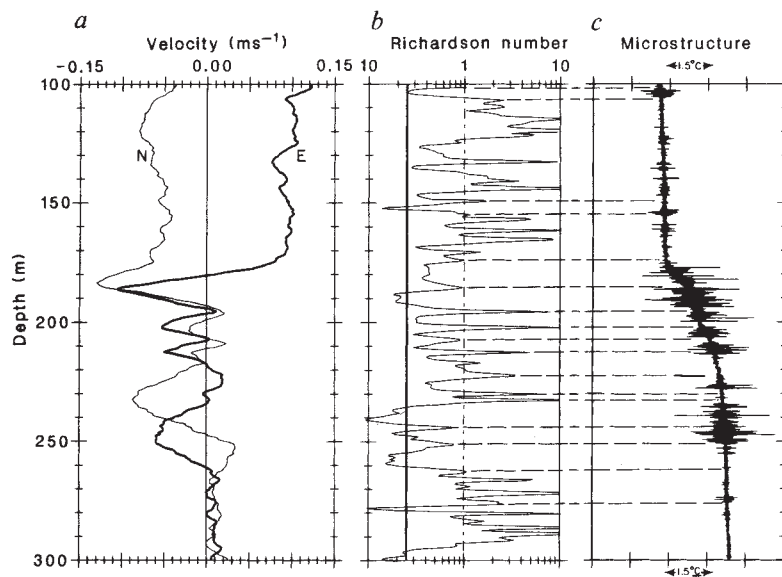


Fig. 3 *a*, An expanded view of the velocity field at the base of the 21°C thermostad obtained from profiler deployment 35. The profile is referenced as in Fig. 2. *b*, The Richardson number profile computed over 3-m vertical scale from least-squares estimates of shear and buoyancy frequency based on 0.5-m vertically averaged temperature, salinity and velocity data. Values of 1/4 and 1.0 are marked with solid and dashed lines respectively. *c*, Profile of raw data from one of the microstructure channels obtained simultaneously with the fine-scale variables used to construct the Richardson number profile. Bursts of small-scale activity mark the zones experiencing turbulent mixing. These data were electronically pre-emphasized to reduce dynamic range before digitization. Consequently, high-frequency variations in the profile scale with vertical temperature gradient, low-frequency changes are inversely proportional to temperature. Horizontal lines are drawn between the Richardson number and microstructure profiles for reference.

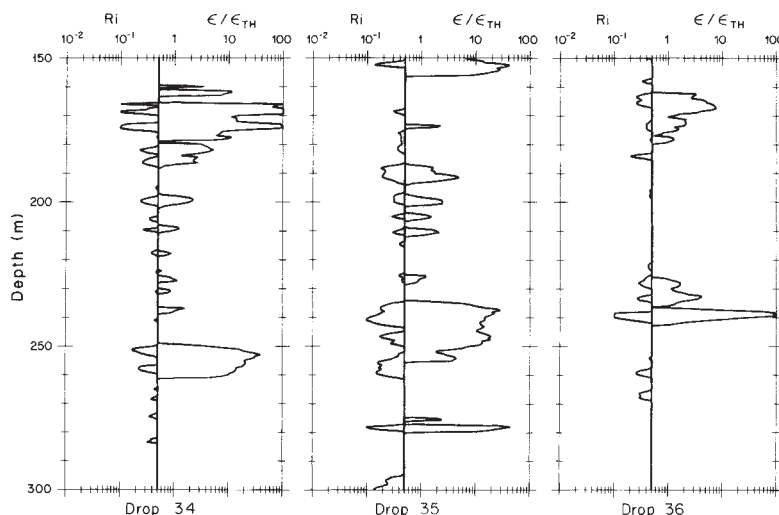
$N^2/(U_z^2 + V_z^2)$ where N is the fluid's buoyancy frequency and U_z and V_z are the vertical gradients of east and north velocity).

The Richardson number at the level of the internal waves noted above shows striking modulation on the vertical scale of the internal waves. High visual correlation is found between the Ri profile and the small-scale temperature gradient field (Fig. 3). As theories¹⁴ and laboratory work¹⁵ suggest, intense patches of microstructure are generally located where Ri is small. It is

also apparent, however, that low Richardson numbers are not universally accompanied by elevated levels of microstructure (consider for example the low Ri feature at 260 m depth in Fig. 3).

Relationships between quantitative measures of the microstructure characteristics and the Ri were also examined. The kinetic energy dissipation rate ($\epsilon = 7.5\nu\langle u_z^2 \rangle$ where ν is the kinematic viscosity and $\langle u_z^2 \rangle$ is one component of the velocity

Fig. 4 Profiles of Ri and a velocity microstructure activity parameter for profiler drops 34, 35 and 36. The left-half of each panel displays $Ri < 0.5$, the right gives the ratio $\epsilon/\epsilon_{TH} > 0.5$ for kinetic energy dissipation rates greater than $4 \times 10^{-10} \text{ W kg}^{-1}$. Turbulence for which ϵ/ϵ_{TH} is greater than 1.0 is believed to effect a vertical buoyancy flux. See text for definitions of ϵ and ϵ_{TH} which were estimated in successive 0.5-m vertical pieces and subsequently smoothed across 3 m. Ri was estimated from 3-m least squares estimates of vertical density and velocity gradients.



shear variance of the turbulent fluctuations) is a measure of the intensity of velocity microstructure¹⁶. Analysis of the velocity microstructure data indicated that at depths where $Ri \leq 0.3$ the kinetic energy dissipation rates exceeded the empirically derived threshold level for 'active' turbulence: turbulence which effects a vertical buoyancy flux¹⁵ ($\epsilon_{TH} = 16\nu N^2$) (Fig. 4). This implies that vertical mixing generally occurred at depths where turbulent energy could be extracted from the fine-scale velocity field.

The relationships between the internal waves creating the low Ri values and the strong velocity and density gradients at the base of the 21 °C thermostat are not known. Apparent wave velocity amplification with compression of vertical scale could be a consequence of the variation of buoyancy frequency along the path of internal wave packets¹⁷. It is also conceivable that the velocity jump contained a critical layer for the vertically propagating waves¹⁸. Both of these processes result in local modifications of the internal wave field, increasing its susceptibility to shear instability. In addition we note that the measurement site was within the anticyclonic shear field of the FASINEX front: thus, near-inertial internal wave trapping processes¹⁹ could also have influenced the observed wave characteristics.

The vertical scale of the low Ri features produced by the internal waves was short, typically 5 m or smaller. The correlation between low Ri and microstructure intensity in the present data set is only discernible because of the high vertical resolution afforded by the new fine- and microstructure profiler. Ri estimated on vertical scales of 10 m and larger tended to be greater than 1.0 and bore little relation to the microstructure. Although an overall correlation between low Ri and high mixing rates is apparent in these data, specific examples can be identified where the relationship is poor. These discrepancies may reflect the time history of the Ri in the slowly evolving internal wave velocity and density fields. Depth intervals which had not yet met the criteria for shear instability, or had only recently achieved it, would not be expected to exhibit elevated levels of microstructure. Older features in which the Ri had once been supercritical might contain a strong, albeit decaying, microstructure signal. Thus, it may prove possible to use the microstructure information to infer the temporal statistics of the oceanic Ri field. This possibility will be explored in the continuing analysis of the FASINEX data set.

Support for the collection and analysis of these data was provided by the Office of Naval Research (ONR) by a contract with the Woods Hole Oceanographic Institution and by a grant from the NSF. Funds to construct the fine- and microstructure profiler were obtained from ONR and the Department of Defense. This funding is gratefully acknowledged. We thank Neil Oakey for assistance in implementing the velocity microstructure measurements and guidance in data processing

techniques and M. Woodgate-Jones for aid in the analysis and preparation of the paper.

Received 6 October 1986; accepted 18 February 1987.

- Garrett, C. J. R. & Munk, W. H. *Deep Sea Res.* **19**, 823-832 (1972).
- Gargett, A. E. & Holloway, G. J. *mar. Res.* **42**, 15-27 (1984).
- Desaubies, Y. & Smith, W. K. *J. phys. Oceanogr.* **12**, 1245-1259 (1982).
- Gargett, A. E. *J. mar. Res.* **42**, 359-393 (1984).
- Moum, J. N. & Osborn, T. R. *J. phys. Oceanogr.* **16**, 1250-1258 (1986).
- Gregg, M. C. in *Internal Gravity Waves and Small-Scale Turbulence: Proc. (eds Muller, P. & Pujalet, R.)* 1-24 (Hawaii Institute of Geophysics, Honolulu 1984).
- Gregg, M. C., D'Asaro, E. A., Shay, T. J. & Larson, N. J. *J. phys. Oceanogr.* **16**, 856-885 (1986).
- Stage, S. A. & Weller, R. A. *Bull. Am. met. Soc.* **66**, 1522-1520 (1985).
- Hayes, S. P., Milburn, H. B. & Ford, E. F. *J. atmos. ocean. Technol.* **1**, 220-236 (1984).
- Osborn, T. R. *J. phys. Oceanogr.* **4**, 109-115 (1974).
- Pollard, R. *Nature* **323**, 433-434 (1986).
- Leaman, K. D. & Sanford, T. B. *J. geophys. Res.* **15**, 1975-1978 (1975).
- Osborn, T. R. & Cox, C. S. *Geophys. Fluid Dyn.* **3**, 321-345 (1972).
- Miles, J. W. *J. Fluid Dyn.* **10**, 496-508 (1961).
- Rohr, J. J., Helland, K. N., Itsweire, E. C. & Van Atta, C. W. in *5th Int. Symp. on Turbulent Shear Flows*, Cornell University, Ithaca, New York, August 7-9, 1985 (ed. Lumley, J. L.) 21-1-22-6 (Pennsylvania State University, 1985).
- See Oakey, N. S. *J. phys. Oceanogr.* **12**, 256-271 (1982).
- Garrett, C. J. R. & Munk, W. H. *Geophys. Fluid Dyn.* **2**, 225-264 (1972).
- Booker, J. R. & Bretherton, F. P. *J. Fluid Mech.* **27**, 513-539 (1967).
- Kunze, E. & Sanford, T. B. *J. phys. Oceanogr.* **14**, 566-581 (1984).

Lead isotope constraints on the origin of Hawaiian basalts

Chu-Yung Chen

Department of Geology, University of Illinois, Urbana, Illinois 61801, USA

The linear Hawaiian-Emperor seamount chain is the best example of intraplate hotspot volcanism. The nature and number of sources involved in generating the Hawaiian basalts have been subjects of great interest¹⁻¹¹. Trace element abundances^{1,4,6} and Sr, Nd^{1-4,6,9} and He¹¹ isotope ratios of the Hawaiian basalts are consistent with a petrogenetic model involving mixing of components from two mantle sources. However, multiple-source models^{2,5,7-10} were proposed to explain the heterogeneous Pb isotope ratios in the Hawaiian basalts. Here I re-evaluate the Pb isotope ratios in the Hawaiian basalts and show that the Pb isotope ratios can also be explained by the mixing of components from two mantle sources, a primitive mantle source and a mid-ocean-ridge basalt (MORB) source. The primitive mantle source is seen as a plume rising from beneath the asthenosphere with Pb, Sr, Nd and He isotope ratios similar to the bulk Earth estimates. The MORB source is envisioned as the wall rock in the lithosphere and asthenosphere which is heterogeneous and has ranges of Pb, Sr, Nd and He isotope ratios similar to those observed in the northern Pacific MORB.

Hawaiian basalts show systematic variations in Sr, Nd and He isotope ratios and trace element abundance ratios (for example, Ba/La, Rb/Sr, La/Ce, La/Yb) with time of eruptions^{1,3,4,6,11}. Basalts which erupted at later stages (alkalic cap and post-erosional stages) are generally more enriched in trace element abundances but have lower Sr and He isotope ratios and higher Nd isotope ratios than basalts erupted at the shield building stage. Such trace element and isotope (Sr, Nd and He) variation trends can be explained by mixing processes involving two mantle sources^{1,3,4,6,11}. However, the Pb isotope ratios in the Hawaiian basalts do not correlate well with other isotope ratios. It is quite clear that more than three isotopically homogeneous components are required to explain the entire Pb, Sr, Nd and He isotope ratios in the Hawaiian basalts^{2,5,7-10}. Three source mixing models have been proposed^{2,5,7-10} and most of these include at least one heterogeneous source^{2,5,7,8,10}. Because a heterogeneous source (multiple component) is required in the multiple-source models^{2,5,7,8,10}, it is important to see if the Hawaiian basalts can be generated by a simpler model such as melting of a single heterogeneous mantle source or two mantle sources, one of which is heterogeneous.

The small-scale isotope heterogeneity of Hawaiian basalts¹⁻¹⁰ and the migration of the volcanoes across the Pacific plate imply that the source of Hawaiian basalts must reside in the moving plate (lithosphere) if these basalts are generated by partial melting of only one mantle source. Melting of the lithosphere to generate Hawaiian basalts has previously been proposed^{12,13}. But experimental studies^{14,15} show that within the lithospheric pressure range (≤ 30 kbar), the nephelinitic and alkalic basalts are generated at a greater depth than the tholeiitic basalts. Based on these studies, the eruption sequences of major Hawaiian volcanoes imply a melting process which migrates downwards in the lithosphere. This is in direct conflict with the bathymetry and heat-flow data which indicate heating below the lithosphere^{16,17}. If Hawaiian basalts are the result of partial melting of a source region restricted largely to the lithosphere, basal heating should first create partial melting near the base of the source region with subsequent melting migrating upwards to shallower depths. Therefore, accounting for the evolution of Hawaiian volcanoes by successive downward melting of a heterogeneous lithosphere is inconsistent with geophysical evidence.

In addition, the volume of an average Hawaiian shield volcano is estimated to be 20,000–45,000 km³ (ref. 18). If Hawaiian tholeiites are generated by 20% partial melting of the mantle source¹⁹, the volume of the tholeiites requires partial melting of a 50-km-diameter (average size of a major Hawaiian volcano) cylinder 50 km to 100 km in depth underlying each Hawaiian volcano. Consequently, melting of most of the underlying lithosphere is required to generate an Hawaiian tholeiitic shield. If the Hawaiian tholeiites are generated by less than 20% of partial melting²⁰, partial melting of even the entire lithosphere is insufficient to generate the Hawaiian basalts. Based on the heat and volume considerations, models involving melting of more than one source are preferred. A model involving mixing of components from a mantle plume and a MORB source, which is consistent with trace element abundances and Sr, Nd and He isotope ratios in the Hawaiian basalts^{1-4,6,9,11} is considered below.

Pb isotope data for Hawaiian basalts^{2,5,7,8,21,22} and MORB²¹⁻²⁶ (summarized in Fig. 1) are essential for understanding the origin of Hawaiian basalts. It has been shown that a global isotope anomaly exists in the Southern Hemisphere²⁷ and that MORBs from different oceans have different isotope characteristics^{25,28}. To eliminate the complexity of regional differences in MORB sources, the MORB data compiled in this paper (Figs 1, 2 and 3) are restricted to those from the northern Pacific Ocean. The $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$ ratios in Hawaiian lavas overlap significantly with those of the northern Pacific MORB (Fig. 1a). However, the $^{208}\text{Pb}/^{204}\text{Pb}$ ratios of most Hawaiian basalts are

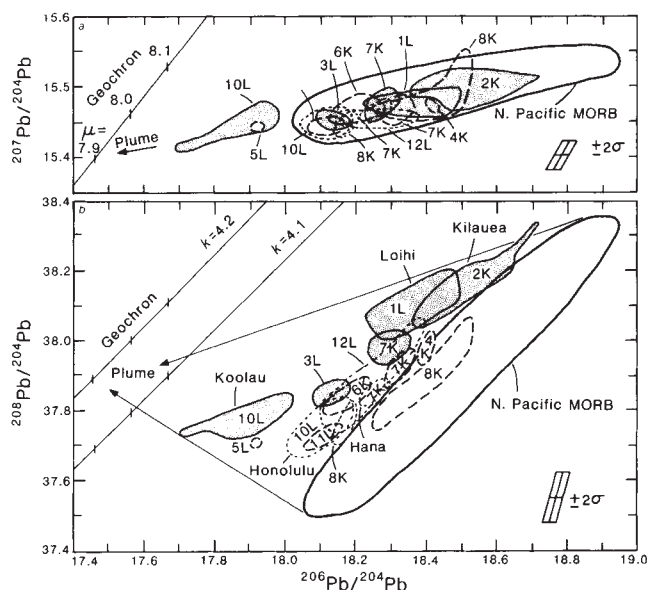


Fig. 1 *a*, The $^{207}\text{Pb}/^{204}\text{Pb}$ ratios against $^{206}\text{Pb}/^{204}\text{Pb}$ ratios. *b*, The $^{208}\text{Pb}/^{204}\text{Pb}$ ratios against $^{206}\text{Pb}/^{204}\text{Pb}$ ratios of Hawaiian basalts^{2,5,7,8,21,22} and MORBs from the northern Pacific Ocean²¹⁻²⁶. Individual volcanoes are marked by numbers: 1, Loihi; 2, Kilauea; 3, Mauna Loa; 4, Mauna Kea; 5, Hualalai; 6, Kohala; 7, Haleakala; 8, West Maui; 9, Molokai; 10, Koolau; 11, Waianae; 12, Kauai. The Hawaiian basalts are divided into three groups according to their stratigraphic positions^{1-8,38}. First, filled areas which include basalts erupted at early shield building stage (Loihi) and main shield building stage (Koolau, Mauna Loa, main Honomanu series of Haleakala and Kilauea). The main shield building stage is characterized by predominately tholeiitic basalt eruptions; second, fields circled by long broken lines. These include samples erupted during late shield building stage which is characterized by interbedded tholeiitic and alkalic basalts or predominately alkalic basalts (Hualalai, Waianae, Kohala, upper Honomanu series of Haleakala, middle and upper Wailuku series of West Maui and Hamaikua series of Mauna Kea) and those erupted at alkalic cap stage (Kula series of Haleakala and Honolulu series of West Maui); third, fields circled by short dashed lines. These fields include samples erupted at the post-erosional stage (Honolulu series of Koolau, Lahaina series of West Maui, Hana series of Haleakala, and Koloa series of Kauai). 'K' and 'L' represent samples from the Kea group and Loa group, respectively. Samples from these two groups overlap significantly. Geochrons for variable μ ($^{238}\text{U}/^{204}\text{Pb}$) and for k ($^{232}\text{Th}/^{238}\text{U}$) = 4.1 and 4.2 are also shown. The arrows in (*a*) and (*b*) are mixing lines pointing to the direction of the plume source. Additional data sources: C.-Y.C. and co-workers, unpublished data for Kilauea basalts; S. R. Hart and C.-Y.C., unpublished data for Haleakala; S. R. Hart, unpublished data for Koolau.

distinct from the northern Pacific MORB values and they plot to the left of the MORB trend in a $^{208}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$ diagram (Fig. 1b). Based on their spatial distributions the Hawaiian volcanoes have been divided into two groups: the Kea group and the Loa group²⁹. It has previously been proposed that volcanoes along the two trends have distinct Pb isotope variations, with basalts from the Kea group having more radiogenic Pb than basalts from the Loa group^{2,5,8,21}. However, Fig. 1a and b shows that samples from the two groups overlap significantly. Samples from these two groups also overlap significantly in $^{87}\text{Sr}/^{86}\text{Sr}$, $^{143}\text{Nd}/^{144}\text{Nd}$ ^{11-18,30,31} (Fig. 2) and $^3\text{He}/^4\text{He}$ ratios^{11,32}. Therefore, lateral lithospheric heterogeneity along the Kea and Loa trends^{2,5,8,10,21} cannot explain all the variations of Pb, Sr, Nd and He isotope ratios among Hawaiian lavas. Figs 1b and 2 show that the evolutionary stage of individual volcanoes is an additional factor that is important in explaining isotope variations of Hawaiian basalts. Although not as pronounced as $^{87}\text{Sr}/^{86}\text{Sr}$, $^{143}\text{Nd}/^{144}\text{Nd}$ (Fig. 2) and $^3\text{He}/^4\text{He}$

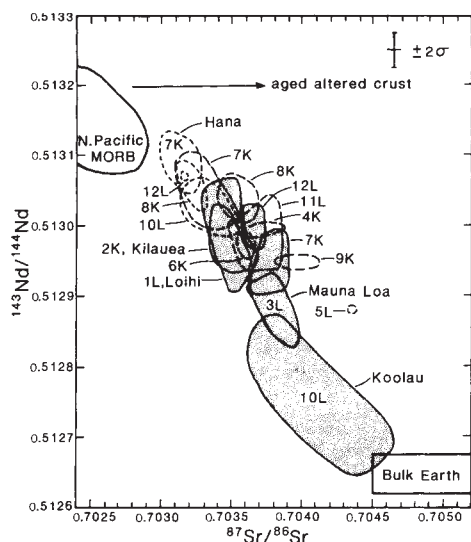


Fig. 2 The $^{143}\text{Nd}/^{144}\text{Nd}$ ratios against $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for Hawaiian basalts^{1-8,30,31}, MORBs from northern Pacific Ocean^{24,25,39,40} and bulk Earth^{1,41}. The arrow indicates the direction of MORB isotope variation due to seawater alteration. Other symbols are as in Fig. 1.

isotope ratios¹¹, the Pb isotope ratios also correlate with the evolutionary stages of the Hawaiian volcanoes (Fig. 1b). Basalts which erupted during the early and main shield building stages have Pb isotope ratios distinct from the MORB values whereas late-stage lavas have Pb isotope ratios approaching MORB values.

Another important observation is that the northern Pacific MORB has a wide range variation in Pb isotope ratios but that the Sr isotope ratios are typical of normal depleted MORB ($^{87}\text{Sr}/^{86}\text{Sr} \leq 0.7030$, Fig. 3). Because of the extremely low concentration of Pb in sea water and the young ages (<2 million years)²¹⁻²⁶ of the ridge basalts compiled in Figs 1 and 3, seawater alteration cannot be the cause of the Pb isotope heterogeneity. The wide range of Pb isotope variation in northern Pacific MORB must reflect isotope heterogeneity in the MORB source. If the Hawaiian basalts are generated by the mixing of components from a mantle plume and the heterogeneous MORB source, the range of $^{206}\text{Pb}/^{204}\text{Pb}$ ratios in Hawaiian basalts require that the plume component lies on the left side of the Pb-Pb correlation diagrams (Fig. 1a and b). Mixing of components from the heterogeneous Pacific MORB source with a plume component which intercept the geochron at $^{238}\text{U}/^{204}\text{Pb}$ ratio (μ) between 7.9 and 8.0 and $^{232}\text{Th}/^{238}\text{U}$ ratio (k) between 4.1 and 4.2 generates the entire range of Pb isotope ratios in Hawaiian basalts (Fig. 1a and b). Such μ and k values are well within the values estimated for the primitive mantle ($\mu = 7$ to 8, $k = 3.7$ to 4.2)^{21,33}. Therefore, the Pb isotope ratios of Hawaiian basalts are consistent with a model involving mixing of components from a primitive mantle plume and a heterogeneous mantle source similar to the northern Pacific MORB source (Fig. 1a and b). The sub-parallel trends of the Hawaiian basalts to the MORB trend in Figs 1b and 3 can be generated if the relative contribution from the plume source and the heterogeneous MORB source are nearly constant for basalts erupted at similar evolutionary stages but different at different evolutionary stages. Basalts from the early and main shield building stages have a significant contribution from the plume source and they plot away from the MORB field (Figs 1-3). As individual volcanoes evolve with time, the supply of the plume material decreases and the contribution from the MORB source (wall-rock of the plume) becomes more significant; therefore, late stage lavas have isotopic compositions closer to MORB (Figs 1-3).

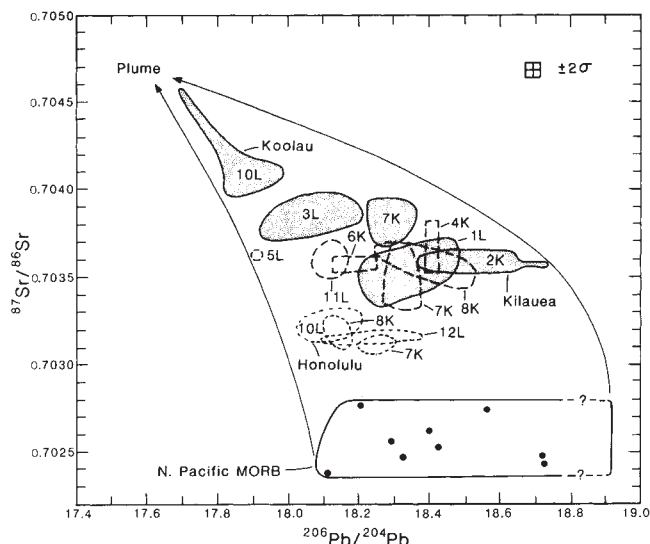


Fig. 3 The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios against $^{206}\text{Pb}/^{204}\text{Pb}$ ratios of Hawaiian basalts¹⁻⁸ and MORBs from the northern Pacific Ocean²⁴⁻²⁶. Lines with arrows pointing to the plume source are two possible mixing lines. The curvatures (R) of these mixing lines require that the (Sr/Pb) ratio in the plume component against the (Sr/Pb) ratio in the MORB component is greater than one. This ($R > 1$) is expected if Pb is more incompatible than Sr³⁴⁻³⁶ and the mixing components from the MORB field are incipient melts^{1,6} of the MORB source. Other symbols are as in Fig. 1.

Additional sources such as aged upper lithosphere^{8,10} or crust⁸, aged altered crust⁷, and subducted ancient crust^{7,10} have been proposed to explain the radiogenic $^{206}\text{Pb}/^{204}\text{Pb}$ ratios in Kilauea basalts. However, because U, Th and Pb are highly incompatible during magmatic processes^{22,34-36}, partial melting and fractionation processes will not change U/Pb and Th/Pb ratios of MORB from its source values^{22,34-36}. Therefore it is very difficult, if not impossible, to distinguish the Pb isotope characteristics between unaltered aged ocean crust⁸ (or upper lithosphere^{8,10}) and the MORB source because they evolved with essentially the same parent/daughter (U/Pb and Th/Pb) ratios. Seawater alteration increases U/Pb and Th/Pb ratios in the MORB^{21,37}; therefore aged altered crust and subducted ancient crust will have more radiogenic Pb isotope ratios than the unaltered crust and the MORB source. However, seawater alteration also increases the $^{87}\text{Sr}/^{86}\text{Sr}$ and Rb/Sr ratios but has little effect on $^{143}\text{Nd}/^{144}\text{Nd}$ and Sm/Nd ratios in the ocean crust³⁰. Therefore, aged altered crust and subducted ancient crust should plot towards high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios on a Nd-Sr correlation diagram (Fig. 2). Although the Kilauea basalts have the most radiogenic Pb isotope ratios, they do not fall to the right of other Hawaiian basalts in the Nd-Sr correlation diagram (Fig. 2). Therefore aged altered crust or subducted ancient crust is not an appropriate source for the radiogenic Pb isotope ratios in the Kilauea basalts. Moreover, oxygen and He isotope ratios are inconsistent with altered crust or subducted ancient crust as significant sources for Hawaiian basalts^{10,11,32}. The northern Pacific MORB source which has a wide range of Pb isotope ratios but a narrow range of Sr isotope ratios (Fig. 3) is a more appropriate source for generating the wide range of Pb isotope ratios in Hawaiian basalts.

In conclusion, a melting process involving mixing of a primitive mantle plume with components from an isotopically heterogeneous mantle source similar to the northern Pacific MORB source is a satisfactory explanation for the origin of Hawaiian basalts. Additional sources such as aged ocean crust and subducted ancient crust are not required. This interpretation is consistent with the Pb isotope ratios as well as the Sr, Nd and He isotope ratios and trace element abundances of the Hawaiian basalts^{1,2,4,6,11}.

I thank S. R. Hart for the use of unpublished data and F. A. Frey, S. R. Hart, R. J. Kirkpatrick and W. P. Chen for valuable comments and informal reviews on the paper. Constructive critical reviews from S. L. Goldstein were appreciated.

Received 6 August 1986; accepted 24 February 1987.

- Chen, C.-Y. & Frey, F. A. *Nature* **302**, 785-789 (1983).
- Stille, P., Unruh, D. M. & Tatsumoto, M. *Nature* **304**, 25-29 (1983).
- Clague, D. A., Frey, F. A. & Beeson, M. H. *Eos* **64**, 902 (1983).
- Roden, M. F., Frey, F. A. & Clague, D. A. *Earth planet. Sci. Lett.* **69**, 141-158 (1984).
- Staudigel, H. et al. *Earth planet. Sci.* **69**, 13-29 (1984).
- Chen, C.-Y. & Frey, F. A. *J. geophys. Res.* **90**, 8743-8768 (1985).
- Li, S. & Hart, S. R. *Eos* **66**, 1133 (1985).
- Hegner, E., Unruh, D. & Tatsumoto, M. *Nature* **319**, 478-480 (1986).
- Feigenson, M. D. *J. geophys. Res.* **91**, 9383-9393 (1986).
- Stille, P., Unruh, D. M. & Tatsumoto, M. *Geochim. cosmochim. Acta* **50**, 2303-2319 (1986).
- Kurz, M. D., O'Brien, P. A., Garcia, M. O. & Frey, F. A. *Eos* **66**, 1120 (1985).
- Lanphere, M. A., Dalrymple, G. B. & Clague, V. A. *Init. Rep. DSDP Leg 55*, 695-706 (1980).
- Wright, T. L. *J. geophys. Res.* **89**, 3233-3252 (1984).
- Yoder, H. S. & Tilley, C. E. *J. Petrology* **3**, 342-352 (1962).
- Kushiro, I. *Tectonophysics* **17**, 211-222 (1973).
- Crough, S. T. *Geophys. J. R. astr. Soc.* **55**, 451-469 (1978).
- Menard, H. W. & McNutt, M. W. *J. geophys. Res.* **87**, 8570-8580 (1982).
- Bargar, K. E. & Jackson, E. D. *J. Res. U.S. Geol. Survey*, **2**, 545-550 (1974).
- Ringwood, A. E. *Composition and Petrology of the Earth's Mantle* (McGraw-Hill, New York, 1975).
- McKenzie, D. P. *J. Petrology* **25**, 713-765 (1984).
- Tatsumoto, M. *Earth planet. Sci. Lett.* **38**, 63-87 (1978).
- Sun, S. S. *Phil. Trans. R. Soc. A* **297**, 409-445 (1980).
- Church, S. E. & Tatsumoto, M. *Contr. Miner. Petrology* **53**, 253-279 (1975).
- Cohen, R. S., Evensen, N. M., Hamilton, P. J. & O'Nions, R. K. *Nature* **283**, 149-153 (1980).
- Cohen, R. S. & O'Nions, R. K. *J. Petrology* **23**, 299-324 (1982).
- Hamelin, B., Dupre, B. & Allegre, C. J. *Earth planet. Sci. Lett.* **67**, 340-350 (1984).
- Hart, S. R. *Nature* **28**, 753-757 (1984).
- Hamelin, B., Dupre, B. & Allegre, C. J. *Earth planet. Sci.* **76**, 288-298 (1986).
- Jackson, E. D., Silver, E. A. & Dalrymple, G. B. *Geol. Soc. Am. Bull.* **83**, 601-618 (1972).
- O'Nions, R. K., Hamilton, P. J. & Evensen, N. M. *Earth planet. Sci. Lett.* **34**, 13-22 (1977).
- White, W. M. & Hofman, A. W. *Nature* **296**, 821-825 (1982).
- Rison, W. & Craig, H. *Earth planet. Sci. Lett.* **66**, 407-426 (1983).
- Allegre, C. J., Dupre, B. & Lewin, E. *Chem. Geol.* (submitted).
- Watson, E. B., Ben Othman, D., Luck, J. M. & Hofman, A. W. *Terra Cognita* **5**(1), 276 (1985).
- Hofmann, A. W., Jochum, K. P., Seufert, M. & White, W. M. *Earth planet. Sci.* **79**, 33-45 (1986).
- Newson, H. E., White, W. M., Jochum, K. P. & Hofmann, A. W. *Earth planet. Sci.* **80**, 299-313 (1986).
- Hamelin, B., Dupre, B. & Allegre, C. J. *Earth planet. Sci. Lett.* **67**, 351-366 (1984).
- Macdonald, G. A. & Katsura, T. *J. Petrology* **5**, 82-133 (1964).
- Zindler, A., Staudigel, H. & Batiza, R. *Earth planet. Sci. Lett.* **70**, 175-195 (1984).
- Macdougall, J. D. & Lugmair, G. W. *Earth planet. Sci. Lett.* **77**, 273-284 (1986).
- Jacobsen, S. B. & Wasserburg, G. J. *J. geophys. Res.* **84**, 7411-7427 (1979).

Extrapolation of the kinetics of oil and gas formation from laboratory experiments to sedimentary basins

P. Ungerer & R. Pelet

Institut Français du Pétrole, 1-4, Avenue de Bois Préau, 92506 Rueil Malmaison Cedex, France

It is generally accepted that the formation of oil and gas from sedimentary organic matter—or kerogen—is adequately described by kinetic models based on first-order kinetics with rate constants obeying an Arrhenius equation. These models, which are extensively used in oil exploration to assess the petroleum potential of sedimentary basins, are based on geological observations^{1,2,3,4,5,6}, but are formally quite similar to models designed to simulate oil-shale pyrolysis^{7,8,9} or coal processing^{10,11,12}. The idea of blending these two approaches is not new (see, for example, ref. 2), but until now the most sophisticated attempts reproduced only the gross features of kerogen cracking on a geological timescale⁵ or were uncontrolled by geochemical measurements^{13,14}. Here we show that the kinetic parameters determined from the laboratory pyrolysis of an immature kerogen sample apply to the conditions of sedimentary basins, although the time and temperature scale are completely different.

Deriving the kinetic parameters with pyrolysis experiments only, brings considerable advantages compared with previous models^{2,4}. Indeed, with laboratory experiments, time, temperature and effluent quantities are precisely known. This is not

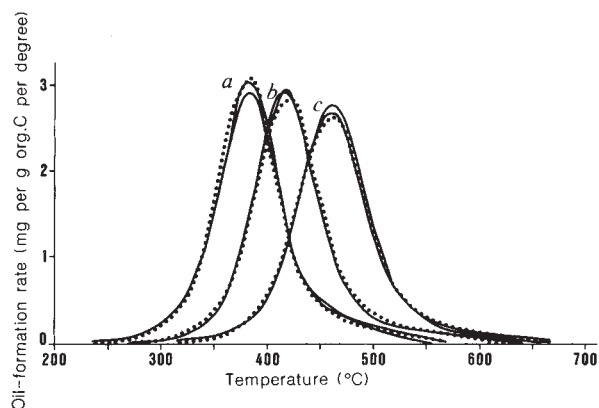


Fig. 1 Experimental pyrolysis curves (—) obtained with a type III (continental origin) Tertiary Miocene coal of the Mahakam Delta, Indonesia, compared with model results (...) at three different heating rates (*a*, 0.34; *b*, 0.45 and *c*, 56 K min⁻¹). Each of the experimental curves is repeated to ascertain reproducibility. The rates of hydrocarbon release are expressed in milligram per gram per kelvin, so that the curves recorded at various heating rates appear with comparable sizes.

so for samples matured in geological conditions. The reconstruction of their time-temperature history cannot be done with any certainty, and their hydrocarbon content may have been influenced by migration. Finally, compared with the theoretical computation of kinetic parameters^{15,16}, the kinetic interpretation of laboratory experiments does not require the knowledge of every reaction of concern, which is unavailable.¹⁷

Conceptually our model is Tissot's model¹, which considers *N* parallel reactions obeying first-order kinetics, with rate constants following an Arrhenius equation:

$$dX_i/dt = -A_i X_i \exp(-E_i/RT) \quad \text{for } i = 1 \dots N \quad (1)$$

where *t* is time, *T* is temperature, *X_i* is the residual potential of oil and gas formation associated to reaction *i*, *R* is the molar gas constant and *A_i* is the Arrhenius factor.

The amount *Q* of oil and gas formed is expressed by:

$$Q = \sum_{i=1}^N (X_{i0} - X_i) \quad (2)$$

where *X_{i0}* is the value of *X_i* at *t* = 0.

When interpreting a given experiment, no attempt is made to obtain analytical integrals for *X_i* (they exist only for the isothermal case), and integrations are performed numerically. This enables us to introduce the actual, measured temperature law, whatever its detailed form.

This formulation is interpreted as follows. The set of possible reactions is finite, but unknown, and because of the highly disordered structure of kerogen¹⁷, each individual bond has its own environment resulting in a final continuum of bond energies. The partition *i* = 1, ..., *N* is an arbitrary digitization of this continuum, and the aim of the optimization is to determine what fraction *X_{i0}* of the original kerogen is to be affected by each reaction. This fraction *X_{i0}* is not directly related to the concentration of a given type of chemical bond (such as C—C, C=C, C—O), which could be measured by infrared spectrometry or by nuclear magnetic resonance for instance. Indeed, a given bond type has different activation energies depending on its environment and may release components of variable molecular mass when cracked.

For these calculations, the activation energies *E_i* are regularly spaced with an interval of 2 kcal mol⁻¹ between 40 and 80 kcal mol⁻¹. This range has been found sufficient to account for the thermal-cracking kinetics of the various types of kerogen that we have studied. As already proposed for oil shales⁹ or coals¹², the Arrhenius factor *A_i* has been considered constant for every *i*, but this unique value is a free parameter for the optimization.

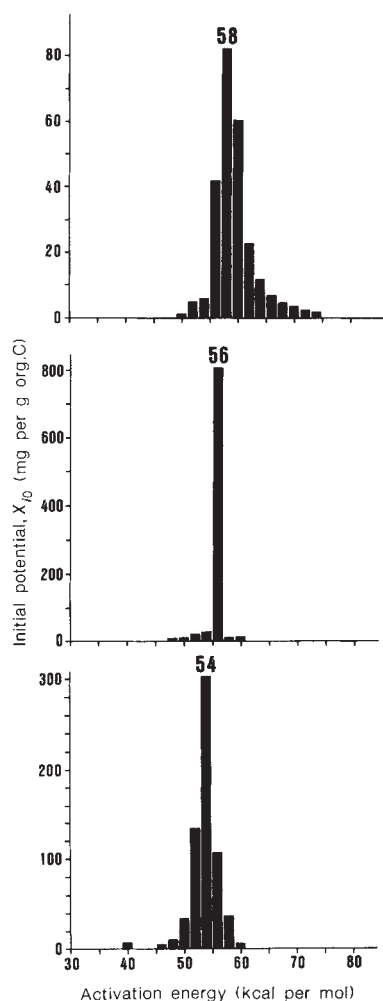


Fig. 2 Distribution of the elementary activation energies found by optimization with reference to pyrolysis data on kerogens of the three major types. *a*, Type III (continental origin) Tertiary coal of the Mahakam Delta, Indonesia. This broad and dissymmetric distribution, obtained from the data of Fig. 1, exhibits a tail up to 70 kcal mol⁻¹, which is characteristic of kerogens of continental origin. Total potential: $\Sigma X_{i0} = 250$ mg g⁻¹ organic C. *b*, Type I (Lacustrine origin) Green River Shales, Uinta Basin, USA (Eocene). The very narrow distribution, with a mean value slightly lower than for continental kerogens is typical of a lacustrine origin. Total potential: $\Sigma X_{i0} = 898$ mg g⁻¹ organic C. *c*, Type II (marine origin) Lower Toarcian Shales, Paris Basin, France. Such a relatively narrow distribution, centred on values lower than the two other types, is generally associated with organic matter of marine origin deposited in good conditions of preservation. Total potential: $\Sigma X_{i0} = 630$ mg g⁻¹ organic C.

In practical terms, this forces the elementary reactions to occur in the same successive order whatever the temperature conditions, the lower activation energies being consumed first. This is supported by the observation that the major steps in hydrocarbon formation take place in the same order in experimental simulation as in sedimentary basins¹⁸. Taking a single A_i value is justified by the limited variation of the Arrhenius factor, which lies generally between 10^{14} and 10^{18} s⁻¹ for unimolecular cracking reactions^{15,19}. Such a variation has, in fact, a low influence on the rate constants $A_i \exp(-E_i/RT)$ compared with their range of variation under the influence of temperature or activation energy. As a consequence, $N+1$ parameters are optimized, namely the initial concentrations X_{i0} and the Arrhenius constant A . In its present state, this model represents the primary cracking reactions of kerogen only (that is, reactions that yield thermally extractable products) disregarding the secondary reactions of these products themselves. Consequently, it cannot forecast the

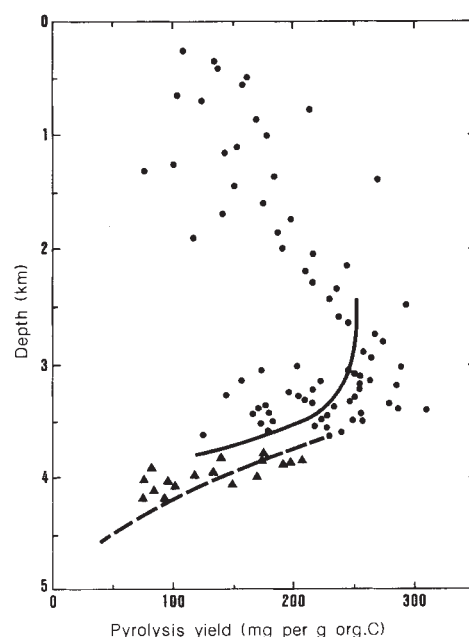


Fig. 3 Standard Rock-Eval pyrolysis yields for coals of the Kerbau wells, Mahakam delta²⁶ compared with the predictions of the kinetic model of Fig. 2*a*. The discontinuities are caused by different thermal régimes in the wells used for the study. The decrease of pyrolysis yields below 2,500 m depth indicates the zone of oil and gas formation. The increase from 0 to 2,500 m is not reproduced by the model, which does not account for loss of CO₂ that is considered responsible for it²⁰. Measurements: ●, Kerbau 1, 3, 4; ▲, Kerbau 2. Model: —, Kerbau 1, 3, 4; ---, Kerbau 2.

detailed composition of these products. As the laboratory calibration is performed on hydrocarbons only, it cannot account for CO₂, H₂O, H₂S and so on, which are mostly generated before the formation of oil and gas²⁰.

After isolation²¹ kerogen samples are introduced in the cell of a standard Rock-Eval pyrolysis device^{22,23} fitted with an additional thermocouple to monitor the true temperature of the sample. The reaction products are swept by a helium gas flow and burnt in a flame ionization detector (FID) online, which delivers a signal (Fig. 1) representing the rate of release of the effluent moieties against temperature (dQ/dt in equations (1) and (2)). For a given sample, three heating rates are used (0.34, 4.5 and 56 K min⁻¹) and the corresponding data are processed by a computer program that determines A and X_{i0} so as to minimize an error function. This error function is obtained by summation of the quadratic differences between the computed and observed rates of release of oil and gas. The optimization, which is nonlinear, is carried out by a gradient method with variable step²⁴ and is repeated with various random values of (A , X_{i0}) in case of several local minima. A satisfactory reproducibility and reliability requires that the higher and lower heating rates differ by two orders of magnitude at least.

After optimization, the detailed features of the pyrolysis curves are satisfactorily reproduced by the model, as shown in Fig. 1, and the Arrhenius factor A is found in the expected range of 10^{14} – 10^{18} s⁻¹ mentioned above. The X_{i0} versus activation energy plot is a chemical characterization of the kerogen. Such diagrams are given (Fig. 2) for the three main types of organic matter of Tissot *et al.*²⁰. For coal, the dominant activation energies (55–65 kcal mol⁻¹) are also consistent with similar models¹² or more theoretical ones¹⁶. For lacustrine kerogen, the main activation energy and (54 or 56 kcal mol⁻¹ depending on the sample) is close to the 52.8 kcal mol⁻¹ of Burnham and Braun²⁵.

In the Mahakam Delta, Indonesia, the formation of oil and gas can be seen (Fig. 3) from the decreasing trend of pyrolysis

yields measured on samples from increasing depths²⁶. Compared with the amounts of extractable hydrocarbons, this parameter offers the advantage of being undisturbed by hydrocarbon migration, which is important in the area²⁷. To apply the above kinetic model to this basin, we reconstructed the thermal history of various strata in the Kerbau wells from a numerical model of non-steady-state heat transfer within the sediments²⁸. This reconstruction considered regional heat flow data²⁹ and was checked on the present-day temperatures recorded by formation tests. For each depth, the petroleum potential (sum of X_i in (1)) was calculated from the reconstructed time-temperature history and the kinetic constants of Fig. 2b introduced in (1). The results appear to reproduce the natural trends of pyrolysis yields at depths >2500 m. The model accounts also qualitatively for the increase of the peak temperature $T_{\max}$ measured with the Rock-Eval method on samples of increasing depths, providing thus an additional control of the model³. Similar extrapolations of the model have been performed in other sedimentary basins (Uinta Basin, USA; Paris Basin, France; northern North Sea, Norway), resulting in the same agreement with geochemical data, especially when the uncertainty of palaeotemperature reconstruction is considered.

To summarize, the kinetic model presented here reproduces the detailed features of the various types of kerogens studied. The same model, with the same set of numerical constants calibrated on laboratory pyrolysis only, reproduces as well the evolution of the petroleum potentials in sedimentary basins. This extrapolation over eight orders of magnitude in time and about 300 K in temperature must be considered as a validation of the model. Besides, it provides a key tool for the oil industry, enabling a more accurate evaluation of source rock maturity at an early stage of basin exploration, when few wells have been

drilled deep enough to sample the oil-producing source rocks.

We thank J. L. Oudin (Total) for providing rock samples and well data and G. Eglinton (University of Bristol) and G. D. Abbott (Newcastle University) for advice on manuscript.

Received 1 September 1986; accepted 2 January 1987.

1. Tissot, B. P. *Revue Inst. fr. Pétrole* **24**, 470–501 (1969).
2. Tissot, B. P. & Espitalié, J. *Revue Inst. fr. Pétrole* **30**, 743–777 (1975).
3. Ungerer, P. in *Thermal Modeling in Sedimentary Basins* (ed. Burrus, J.) 531–546 (Technip, Paris, 1986).
4. MacKenzie, A. S. & Mackenzie, D. P. *Geol. Mag.* **120**, 417–528 (1983).
5. Sweeney, J. J., Burnham, A. K. & Braun, R. L. in *Thermal Modeling in Sedimentary Basins* (ed. Burrus, J.) 547–561 (Technip, Paris, 1986).
6. Hood, A., Gutjahr, C. C. & Heacock, R. L. *Bull. Am. Ass. Petrol. Geol.* **59**, 986–996 (1975).
7. Allred, V. D. *Chem. Engng Prog.* **62**, 55–60 (1966).
8. Braun, R. L. & Rothman, A. J. *Fuel* **54**, 129–131 (1975).
9. Campbell, J. H., Gallegos, G. & Gregg, M. *Fuel* **59**, 727–732 (1980).
10. Pitt, G. J. in *Proc. 4th Int. Conf. Coal Sci. Le Touquet, France*, 30 May–2 June (1961).
11. Juntgen, H. & Van Heek, K. H. *Fuel* **48**, 103–117 (1968).
12. Juntgen, H. & Klein, J. *Erdöl Kohle Erdas Petrochem.* **28**(2), 65–73 (1975).
13. Akihisa, K. J. *Japan Ass. Petrol. Technol.* **44**, 26–33 (1979).
14. Lewan, M. D. *Phil. Trans. R. Soc. A* **315**, 123–132 (1985).
15. Benson, S. W., *Thermochemical Kinetics*, 2nd edn (Wiley, New York, 1976).
16. Gavalas, G. R., Cheong, P. H.-K. & Jain, R. *Ind. Engng Chem. Fundam.* **20**, 113–122; 122–132 (1981).
17. Behar, F. & Vandenbroucke, M. *Revue Inst. fr. Pétrole* **41**, 173–188 (1986).
18. Monthieux, M., Landais, P. & Durand, B. *Org. Geochem.* **8**, 275–292 (1985).
19. Frost, A. A. & Pearson, R. G. *Kinetics and Mechanism*, 2nd edn (Wiley, New York, 1961).
20. Tissot, B. P. & Welte, D. H. *Petroleum Formation and Occurrence*, 2nd edn (Springer, Berlin, 1984).
21. Durand, B. & Nicaise, G. in *Kerogen, Insoluble Organic Matter from Sedimentary Rocks* (ed. Durand, B.) 35–53 (Technip, Paris, 1980).
22. Espitalié, J., Laporte, J. L., Madec, M., Marquis, F., Leplat, P. & Paulet, J. *Revue Inst. fr. Pétrole* **32**, 23–43 (1978).
23. Espitalié, J., Deroo, G. & Marquis, F. *Revue Inst. fr. Pétrole* **40**, 755–784 (1985).
24. Ciarlet, P. G. *Introduction à l'analyse matricielle et à l'optimisation* (Masson, Paris, 1982).
25. Burnham, A. K. & Braun, R. L. *In situ* **9**, 1–23 (1985).
26. Huc, A. et al. *Advances in Organic Geochemistry 1985; Org. Geochem.* **10**, 65–72 (1986).
27. Vandenbroucke, M., Durand, B. & Oudin, J. L. in *Advances in Organic Geochemistry 1981* (ed. Bjoroy, M.) 147–155 (Wiley, New York, 1983).
28. Ungerer, P. et al. *Mem. Am. Ass. Petrol. Geol.* **35** (eds Demaison, G. & Murriss, R. J.) 53–77 (1984).
29. Vacquier, V. *Tectonophysics* **103**, 81–98 (1984).

The exaerobic zone, a new oxygen-deficient marine biofacies

Charles E. Svrda* & David J. Bottjer†

* Department of Geology, Auburn University, Auburn, Alabama 36830, USA

† Department of Geological Sciences, University of Southern California, Los Angeles, California 90089-0741, USA

Classical biofacies models^{1,2} for reconstructing palaeoenvironments of strata deposited in oxygen-deficient marine settings define three principal facies: aerobic (>1.0 ml l⁻¹ O₂), dysaerobic (1.0 to 0.1 ml l⁻¹ O₂) and anaerobic (<0.1 ml l⁻¹ O₂) zones. These models have postulated a decrease in organism size and degree of calcification as well as a drastic reduction in the relative percentage of fauna possessing calcified skeletons as the dysaerobic/anaerobic boundary is approached. Through use of evidence independent of that provided by body fossils, we demonstrate here that in portions of the Monterey Formation (Miocene; California) the bivalve *Anadara montereyana* occurs *in situ* almost exclusively in strata deposited at the dysaerobic/anaerobic boundary. The occurrence of fossils of large well-calcified benthic macroinvertebrates at this redox boundary contradicts classical biofacies models^{1,2} and therefore provides the basis for definition of a new oxygen-related biofacies, the 'exaerobic zone'. The exaerobic zone concept, when adjusted for differences in basin configuration and palaeoceanographic conditions, provides a potentially useful model for explaining occurrences of shelly benthic fossils within laminated, organic-rich strata of other Phanerozoic marine sequences.

Independent evidence for the reconstruction of palaeo-oxygen conditions is provided by the application of a trace fossil model. This refined approach, described in detail elsewhere³, uses observations on general sedimentary fabric (laminated versus bioturbated) that typically have been employed to determine

the dysaerobic/anaerobic boundary^{1,2}, but also incorporates into a trace fossil tiering framework trends of decreasing trace fossil diversity, burrow size and burrow penetration depth with decreasing oxygen availability. The trace fossil model has been used to analyse a short stratigraphic section of the late Miocene Canyon del Rey member of the Monterey Formation exposed along Toro Road in Monterey County, California. This section consists of friable, lithologically homogeneous diatomaceous strata that were deposited at upper bathyal depths (150–500 m)⁴. The distribution of bioturbated and laminated intervals and associated trace fossil types for a one-metre interval of the section is schematically illustrated in Fig. 1. As is characteristic of most of the Monterey Formation, diversity of trace fossil types is very low; *Chondrites* either occurs alone or with and cross-cutting *Planolites*. The interpreted relative oxygenation curve for this section (Fig. 1) is based on general sedimentary fabric, trace fossil assemblage composition and burrow size (as outlined in ref. 3). The curve is constructed with only two qualitative reference lines; (1) the L-line represents the dysaerobic/anaerobic boundary, the threshold below which laminations were preserved and above which *Chondrites*-producing organisms were able to survive, and (2) the P-line represents oxygen levels required to permit occupation of substrates by relatively larger *Planolites*-producing organisms. The relative degree of oxygenation between reference lines is interpreted on the basis of maximum burrow diameter.

The distribution of preserved body fossils in this section demonstrates strong associations with the inferred palaeo-oxygenation curve. Laminated, anaerobic intervals are for the most part unfossiliferous, containing only fish scales and uncommon thin-shelled pectenid bivalve fragments whereas bioturbated intervals that indicate low to moderate oxygenation are characterized by common, interspersed poorly preserved casts and moulds of thin-shelled 'paper pectens' (probably *Delectopecten* and/or *Cyclopecten*) and very rare small specimens of the bivalve *Anadara montereyana*, as well as other unidentified

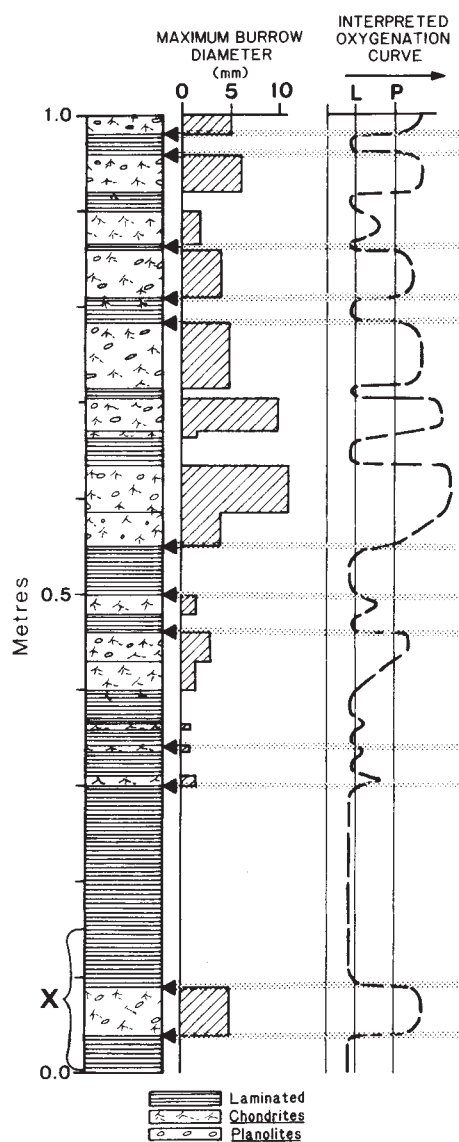


Fig. 1 Presentation of data from high-resolution vertical sequence analysis of a section of the Monterey Formation located in unit 20 of the Toro Road section described in ref. 4. General sedimentary rock fabric types and trace fossil assemblage composition, illustrated schematically in the column, have been used in conjunction with burrow size data to construct the interpreted relative oxygenation curve using the model described in ref. 3. Arrows and stippled bars indicate locations of horizons characterized by dense, large *A. montereyana*, all of which occur at transitions between anaerobic and more oxygenated strata. Interval at 'X' is shown in Fig. 2a.

bivalves and brachiopods. Shell lengths in these assemblages rarely exceed 2 cm. The most striking relationship is the occurrence of abundant, larger specimens of *A. montereyana* (maximum length = 5 cm; maximum height = 3.5 cm) on bedding planes that are coincident with dysaerobic/anaerobic biofacies boundaries (Fig. 1). These bivalves occur with the sagittal plane parallel to bedding planes in densities as high as 4 to 5 specimens per 0.1 m^2 (Fig. 2), with preservation in the form of casts and moulds. Although no original shell material remains, well-developed ribs indicate that these originally aragonitic shells were robust.

All available evidence indicates that these bivalves are *in situ*. Both valves of all specimens of *A. montereyana* at these horizons are intact. Any disarticulation is minor and occurred as a result of compactional slip before the shells were dissolved (Fig. 2). This, combined with the lack of any sedimentological evidence for rapid deposition, indicates that these bivalves were not

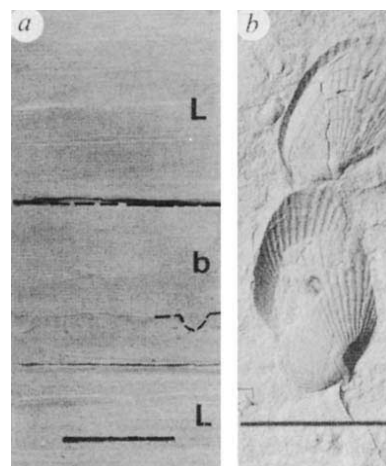


Fig. 2 a, Vertical section of block sample from interval 'X' (Fig. 1) showing the transitions between anaerobic, laminated strata (L) and bioturbated (*Chondrites*, *Planolites*) strata (b). b, Large and small *A. montereyana* on bedding plane surface from the upper transition shown in a. Scale bars, 5 cm.

transported and redeposited from shallower, more oxygen-rich benthic environments. Furthermore, occurrence of *A. montereyana* at stratigraphic locations indicating overall environmental change from both dysaerobic to anaerobic, as well as anaerobic to dysaerobic, conditions (Fig. 1), in addition to functional morphological evidence, demonstrates that this bivalve was not epiplanktonic.

Considering the above, only two factors remain to explain the distribution of these '*Anadara*' horizons; (1) preferential dissolution and (2) acclimation to very low oxygen conditions characteristic of the dysaerobic/anaerobic boundary. Members of the genus *Anadara* have the morphology of shallow burrowers⁵. It is possible that these normally infaunal *A. montereyana* were forced to the sediment-water interface by a rising critical pore-water redox boundary associated with bottom water deoxygenation and that the record of these bivalves before such an event was destroyed due to enhanced dissolution related to biological reworking of pre-anaerobic event sediments⁶. However, the occurrence of dense bedding plane accumulations of *A. montereyana* at transitions from both dysaerobic to anaerobic and anaerobic to dysaerobic conditions precludes a preferential preservation explanation. We can only conclude that these large bivalves lived on the sea floor, within a very narrow depth range coincident with the dysaerobic/anaerobic boundary.

Based on predicted relationships between respiration and calcium carbonate skeleton secretion physiology with declining oxygen availability¹, the restricted occurrence of abundant *A. montereyana* at the palaeo-dysaerobic/anaerobic boundary and their near absence in strata deposited under more highly oxygenated bottom waters is anomalous. This suggests that there may be special geochemical and biological factors at this boundary that favour the settlement and growth of certain macroinvertebrates that are able to respire in water with 0.1 to 0.2 ml l^{-1} dissolved oxygen. Although this is not directly analogous to the so-called 'edge effect' or 'hot spots' observed along the boundaries of the modern oxygen minimum zone off central California⁷, it may be similar in that life at the anaerobic/dysaerobic boundary may be facilitated by bacterially mediated increases in nutrient recycling. The critically balanced juxtaposition of oxygenated (albeit poorly) bottom waters with reducing substrate environments at this boundary provides a suitable interface between dissolved oxygen and free sulphides. Such an interface would provide optimal conditions for sulphur-oxidizing bacteria. In similar contemporary interfacing environments, such as deep-sea hydrothermal vent habitats and sewage

outfall areas, certain bivalve molluscs may have high concentrations of sulphur-oxidizing bacteria in their gill structures. It appears as though these associations are symbiotic, providing a chemosynthetic food source for the molluscs⁸⁻¹¹. We suggest that *A. montereyana* may have had a similar symbiotic association with bacteria. The unusual epifaunal habit of *A. montereyana* may have been due to the location of a critical redox boundary at or just below the sediment-water interface, which may have made life in the sediment impossible for these bivalves.

Application of classical biofacies schemes^{1,2} would lead to the misinterpretation that *A. montereyana*-bearing strata were deposited under well-oxygenated conditions. Hence, modification is needed. We propose the term 'exaerobic zone' to indicate the environments near the dysaerobic/anaerobic boundary (0.1 to 0.2 ml l⁻¹ dissolved oxygen) that are characterized by deposition of laminated strata and anomalous occurrences of shelly faunas. Although genuine examples of a modern exaerobic zone have not yet been discovered, various observations on distribution of macroinvertebrates made in recent studies of contemporary oxygen-deficient environments indicate that moderately- to well-calcified organisms may be significant components of benthic faunas where bottom-water oxygenation levels are at the lower boundary of the dysaerobic range¹²⁻¹⁴.

In the Monterey Formation, exaerobic faunas are restricted to very narrow (<1 cm) stratigraphic intervals. This suggests that the exaerobic zone itself was narrowly restricted and that it shifted position dynamically in response to fluctuations in the vertical position and/or intensity of a mid-water oceanic oxygen minimum zone that impinged upon a relatively steep continental margin. The exaerobic concept can be applied to other ancient basins that had markedly different physiographic and palaeoceanographic characteristics. Consider a broad, shallow, relatively flat-bottomed epicontinental seaway characterized by warm bottom waters and high influx and descent of marine and/or terrestrial organic matter. In this setting, oxygen deficiency is more likely to be generated at the sea floor rather than in the mid-water column in response to intense bacterial oxygen consumption in the sediments. During times of diminished circulation, the dysaerobic/anaerobic boundary would rise above the sediment-water interface. During periods of increased bottom-water circulation, it is theoretically possible, with sustained high oxygen consumption rates, to develop a diffusive boundary layer that could result in the stabilization of the dysaerobic/anaerobic boundary directly at the sediment-water interface¹⁵. Temporal fluctuations between these two conditions could provide a much more laterally extensive and stratigraphically thicker record of exaerobic zone deposition that would be reflected by sequences of laminated, organic-rich strata containing calcified epibenthic macroinvertebrate fossils.

As an example, deposition under exaerobic conditions may explain intervals up to several metres thick found in the Posidonienschiefer (Germany; Jurassic) that (1) have a laminated sediment fabric, (2) contain abundant *in situ* valves of the epibenthic bivalve *Posidonia*, and (3) are transitional between laminated strata, which lack this abundance of *Posidonia*, and bioturbated aerobic strata¹⁶⁻¹⁸. The exaerobic zone concept is also compatible with the interpretation that common, large and heavily calcified inoceramid bivalves found in laminated Upper Cretaceous strata of the North American Western Interior are truly *in situ*^{19,20}, and has strong implications regarding interpretations of other faunal elements, principally bivalve molluscs and brachiopods, found in a wide variety of unbioturbated Phanerozoic strata (for example, refs 21-26).

Further study of dysaerobic-anaerobic transitions in modern and ancient environments will allow a more complete evaluation of the exaerobic zone concept. Suitable modern analogues should be sought through combined biological and geochemical studies that focus on these narrow transitions in contemporary oxygen-deficient settings and through further analysis of the oxygen requirements of extant subtidal faunas. In conjunction

with thorough study of other potential occurrences of exaerobic strata in the rock record, such studies will provide a more complete understanding of the biology of macroinvertebrates that can tolerate poorly oxygenated conditions and will increase the potential for more precise palaeoenvironmental interpretations, particularly for laminated strata that contain benthic macroinvertebrate fossils.

We thank D. S. Gorsline, M. L. Droser and C. W. Byers, for helpful comments and criticisms. A portion of this research was supported by an Exxon Centennial Teaching Fellowship awarded to C.E.S. Grants from the Geological Society of America, Sigma Xi and NSF in support of this research are also gratefully acknowledged.

Received 14 November 1986; accepted 6 February 1987.

1. Rhoads, D. C. & Morse, J. W. *Lethaia* **4**, 413-428 (1971).
2. Byers, C. W. *Spec. Publ. Soc. econ. Paleont. Miner.* **25**, 5-17 (1977).
3. Savrda, C. E. & Bottjer, D. J. *Geology* **14**, 3-6 (1986).
4. Govean, F. M. & Garrison, R. E. in *The Monterey Formation and Related Siliceous Rocks of California* (eds Garrison, R. E. et al.) 181-198 (*Spec. Publ. Pacific Section, Soc. econ. Paleont. Miner.*, 1981).
5. Stanley, S. M. *Mem. geol. Soc. Am.* **125** (1970).
6. Aller, R. C. *J. Geol.* **90**, 79-95 (1982).
7. Mullins, H. T. et al. *Geology* **13**, 491-494 (1985).
8. Hand, S. J. & Somero, G. N. *Biol. Bull.* **165**, 167-181 (1983).
9. Felbeck, H. J. *comp. Physiol.* **152**, 3-11 (1983).
10. Dando, A. J. et al. *Mar. Ecol. Prog. Ser.* **23**, 85-98 (1985).
11. Reid, R. G. B. & Brand, D. G. *Veliger*, **29**, 3-24 (1986).
12. Tunncliffe, V. *Nature* **294**, 354-356 (1981).
13. Savrda, C. E., Bottjer, D. J. & Gorsline, D. S. *Bull. Am. Ass. Petrol. Geol.* **68**, 1179-1192 (1984).
14. Thompson, J. B. et al. *Lethaia* **18**, 167-179 (1985).
15. Jorgensen, B. B. & Revsbech, N. P. *Limnol. Oceanogr.* **30**, 111-122 (1985).
16. Kauffman, E. G. in *Communities of the Past* (eds Gray, J., Boucot, A. J. & Berry, W. B. N.) 311-381 (Hutchinson Ross, Stroudsburg, Pennsylvania, 1981).
17. Seilacher, A. *Neues Jahrbuch für Geologie und Paläontologie Monatshefte* 98-114 (1982).
18. Seilacher, A. *Proc. 1st Int. Meet. on Paleontology, Essential of Historical Geology Venice*, 25-55 (1982).
19. Kauffman, E. G. *The Mount. Geologist* **14**, 75-99 (1977).
20. Hattin, D. E. *Kans. geol. Surv. Bull.* **225**, 1-108 (1982).
21. Thayer, C. W. *Lethaia* **7**, 121-155 (1974).
22. Heckel, P. H. *Bull. Am. Ass. Petrol. Geol.* **61**, 1045-1068 (1977).
23. Sheehan, P. M. *Lethaia* **10**, 201-203 (1977).
24. Watkins, R. & Berry, W. B. N. *Lethaia* **10**, 267-286 (1977).
25. Morris, K. A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **26**, 117-126 (1979).
26. Leggett, J. K. *J. geol. Soc. Lond.* **137**, 139-156 (1980).

Discovery of *Nothofagus* fruits corresponding to an important Tertiary pollen type

Robert S. Hill

Botany Department, University of Tasmania, GPO Box 252C, Hobart, Tasmania, Australia 7001

Nothofagus is common in Southern Hemisphere temperate forests today and was even more widespread and dominant during the middle Tertiary¹⁻⁷. Although a type of *Nothofagus* pollen, the *N. brassii*-type, dominated the Southern Hemisphere in the middle Tertiary, there has been no previous macrofossil record. The discrepancy between the abundant *N. brassii*-type pollen record and the lack of a macrofossil record is the major problem in Southern Hemisphere Tertiary vegetation reconstruction. The late Oligocene Little Rapid River flora in north-west Tasmania contains three types of *Nothofagus* cupules. One belongs to subsection *Bipartitae*, which is uniquely associated with the *N. brassii*-type pollen. The second type is similar to cupules of the extant Australian species *N. cunninghamii* and *N. moorei*, and the third has unknown affinities. The presence of taxonomic groups which are now separated latitudinally by about 1,500 km in Tasmanian Tertiary forests supports the hypothesis that early to middle Tertiary climatic conditions in southeastern Australia may have no modern analogue.

Nothofagus is divided into deciduous species (section *Nothofagus*) and evergreen species (section *Calusparassus*), comprising the subsections *Bipartitae* (2-valved cupules), *Tripartitae* (3-valved cupules) and *Quadripartitae* (4-valved

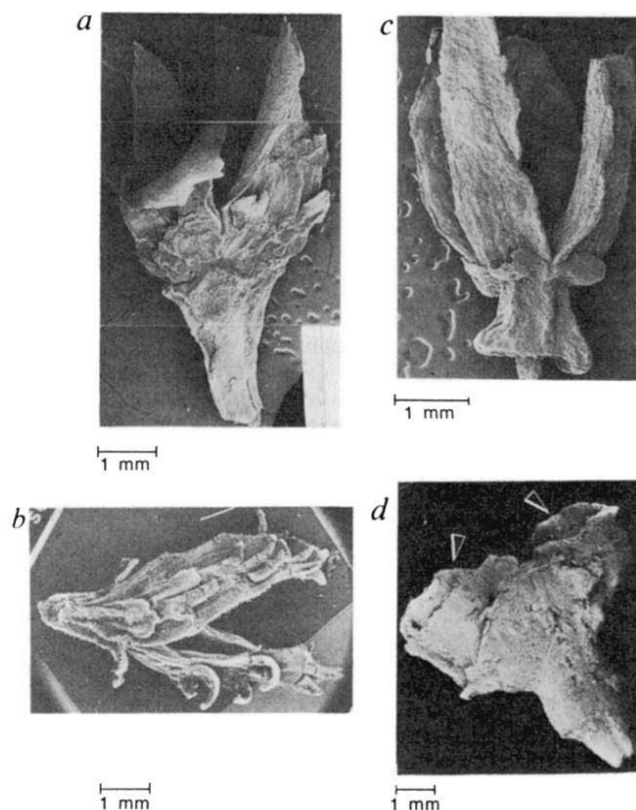


Fig. 1 Scanning electron micrographs of: *a*, a fossil 4-valved cupule similar to the type produced by extant *Nothofagus cunninghamii* and *N. moorei*; *b*, a 4-valved cupule of *N. cunninghamii*; *c*, a fossil 4-valved cupule with a short, thickened peduncle. This cupule type is distinct from those produced by all extant Australian *Nothofagus* species; *d*, a fossil 2-valved cupule. Both valves can be seen (arrowed), as can two lamellae on the left hand cupule valve.

cupules)¹. Palynologists recognize three distinct pollen types, informally classed as *N. brassii*, *N. menziesii* or *N. fusca*^{2,3}. The only correlation between the subgeneric taxonomy and the pollen groupings is that the subsection *Bipartitae* uniquely produces the *N. brassii*-type pollen.

Subsection *Bipartitae* is now restricted to Papua New Guinea, New Britain and New Caledonia, ~1,500 km from *Nothofagus* species in Australia and New Zealand. But Tertiary pollen records from the Southern Hemisphere⁴⁻⁷ indicate that species from subsection *Bipartitae* commonly occurred with other *Nothofagus* species. *Nothofagus* pollen dominated the middle Tertiary of Australia and New Zealand^{4,5}, with the *N. brassii*-type making the major contribution. Towards the end of the Tertiary the *N. brassii*-type pollen was gradually restricted to northern Australia, and eventually became extinct there, but the parent species survive in Papua New Guinea and New Britain. A similar history applies to New Zealand and New Caledonia⁵.

Despite the *N. brassii*-type pollen dominance of middle Tertiary deposits in the Southern Hemisphere, the associated macrofossils are unrecorded. This may be because the plants that now produce the *N. brassii*-type pollen evolved to their present form relatively recently, so that the macrofossils, although present, are unrecognized. *Nothofagus* macrofossils are known from Australia, but they are of the type with 4-valved cupules, and most are related to extant Australian species⁸⁻¹¹.

The Little Rapid River deposit (41°09' S, 145°14' E) in north-west Tasmania is of major significance, as it contains cupules of *Nothofagus* subsection *Bipartitae*. The fluviatile sands are assigned to the Lower *Proteacidites tuberculatus* zone¹², based on the presence of *Cyatheacidites annulatus*, *Chenopodopollis* spp. and *Foveotrilites crater* (taxa whose first appearance define

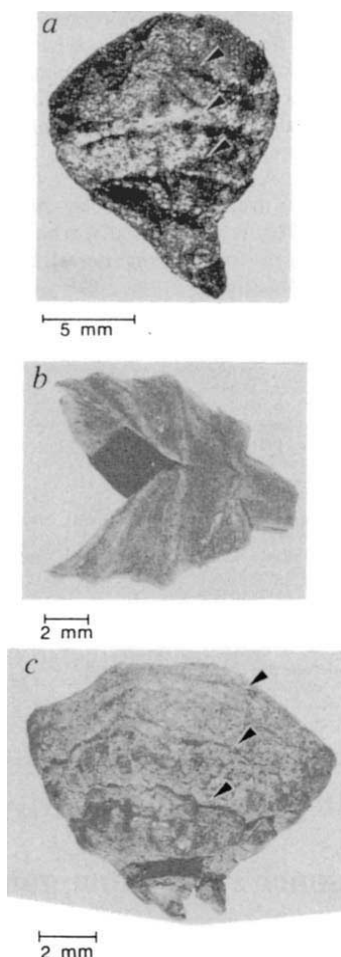


Fig. 2 Reflected light photographs of: *a*, one valve of a fossil 2-valved cupule, with three lamellae (arrows) and a short peduncle visible; *b*, a 2-valved cupule of *N. perryi* from Papua New Guinea with lamellae visible on both cupule valves; *c*, one valve of a 2-valved cupule of *N. grandis* from Papua New Guinea. Three lamellae are arrowed.

the zonule base) and *Nothofagidites flemingii*, *Aglaoreidia qualumis*, *Granodiporites nebulosus* and *Rugulatisporites trophus* (taxa which range no higher than this zonule in the Gippsland Basin). On this basis and from a comparison with other Tasmanian palynofloras, M. K. Macphail (personal communication) suggests a late Oligocene age for the deposit. *Nothofagus* pollen dominates the microflora, with the *N. brassii*-type most common, but with significant amounts of the other types. The macroflora contains a high diversity of gymnosperms and angiosperms, including over 70 specimens representing three distinct types of *Nothofagus* cupules. There are two types of 4-valved cupules, one of which is similar to that produced by the extant Australian species *N. moorei* and *N. cunninghamii* (Fig. 1*a, b*). The second type (Fig. 1*c*) has unknown affinities.

The third cupule type is 2-valved and is similar to several extant species in subsection *Bipartitae* in having 3-4 lamellae on the cupule valves and a straight or curved peduncle of variable size (Figs 1*d, 2a-c*)¹³. The cuticle of the fossil cupules has trichome bases of a type common on fossil and extant *Nothofagus* leaves⁸.

The number and preservation of the fossil cupules suggest that they had a local source. Therefore the vegetation at Little Rapid River probably contained at least three *Nothofagus* species in close proximity. This is the first evidence of the Tertiary co-occurrence, possibly in the same community, of species from subsections *Bipartitae* and *Quadripartitae*. The separation of these subsections by ~1,500 km latitudinally

today, along with the associated differences in climate, community structure and floristics in areas where they occur (J. Read, R.S.H. & G. S. Hope, manuscript in preparation), supports the hypothesis⁷ that early to middle Tertiary climatic conditions in southeastern Australia may have no modern analogue. The co-occurrence of these species in the middle Tertiary may also reflect a more unstable Australian landscape then, as Australia is now one of the few areas inhabited by *Nothofagus* which does not experience recurrent land disturbance^{14,15}. Regular disturbance is important for the regeneration of *Nothofagus* species in many communities¹⁶.

I thank the Australian Research Grants Committee for support.

Received 5 December 1986; accepted 27 February 1987.

- Humphries, C. J. in *The Evolving Biosphere* (ed. Forey, P. L.) 283–297 (British Museum (Natural History), London, 1981).
- Cranwell, L. M. *Rec. Auckland Inst. Mus.* 2, 175–196 (1939).
- Cookson, I. C. & Pike, K. M. *Aust. J. Bot.* 3, 197–206 (1955).
- Martin, H. A. *Alcheringa* 2, 181–202 (1978).
- Mildenhall, D. C. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 31, 197–233 (1980).
- Archangelsky, S. & Romero, E. J. *Bol. Soc. Bot. Mexico* 33, 13–30 (1974).
- Kemp, E. M. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 24, 169–208 (1978).
- Hill, R. S. *Aust. J. Bot.* 31, 453–465 (1983).
- Hill, R. S. *Alcheringa* 8, 81–86 (1984).
- Christophel, D. C. *Proc. R. Soc. Vic.* 97, 176–178 (1985).
- Hill, R. S. *Alcheringa* (in the press).
- Stover, L. E. & Partridge, A. D. *Proc. R. Soc. Vic.* 85, 237–286 (1973).
- Soepadmo, E. *Flora Malesiana Series I*, 7, 265–403 (1972).
- Read, J. & Hill, R. S. *Vegetatio* 63, 67–78 (1985).
- Read, J. & Hill, R. S. *New Phytol.* 101, 731–742 (1985).
- Veblen, T. T., Donoso, C. Z., Schlegel, F. M. & Escobar, B. R. *J. Biogeogr.* 8, 211–247 (1981).

No pure strategy is evolutionarily stable in the repeated Prisoner's Dilemma game

Robert Boyd* & Jeffrey P. Lorberbaum†

* Department of Anthropology, University of California, Los Angeles, California 90024, USA

† Department of Systems Science and Mathematics, Washington University, St Louis, Missouri 63130, USA

A knowledge of the conditions under which natural selection can favour cooperative behaviour among unrelated individuals is crucial for understanding the evolution of social behaviour, particularly among humans and other social mammals. In an influential^{1–5} series of works, Axelrod^{6–10} has argued that reciprocal cooperation is likely to evolve when individual organisms interact repeatedly. This conclusion is based, in part, on an evolutionary analysis of the repeated Prisoner's Dilemma game which indicates that strategies which lead to reciprocal cooperation are evolutionarily stable^{1,11}. In this paper, however, we argue that no pure strategy can be evolutionarily stable in this game. This fact casts doubt on several of Axelrod's conclusions about the evolution of reciprocity.

In Axelrod's model, pairs of individuals are sampled from a population and interact repeatedly. The probability that a given pair interacts more than t times is w^t , where $0 < w < 1$. During each interaction individuals choose between cooperation and defection with the incremental effects on the fitness of each individual (T , R , P and S) shown in Table 1. Each individual is further characterized by a strategy which specifies whether it will cooperate or defect during an interaction depending on the sequence of interactions up to that point. Strategies can be fixed rules such as always defect (ALLD), or contingent ones such as TIT FOR TAT (TFT) which cooperates on the first move, and then adopts the behaviour used by the other player during the previous interaction.

Axelrod concludes that when w is sufficiently large, strategies such as TIT FOR TAT which lead to cooperative reciprocity

Table 1 Payoff matrix for the single period Prisoner's Dilemma game

Choice of player 1	Choice of player 2	
	Cooperate	Defect
Cooperate	R, R	S, T
Defect	T, S	P, P

Each cell shows the payoffs to the first player and to the second player, separated by commas, of the pair of choices represented by that cell. The dilemma exists if $T > R > P > S$ and $R > (T + S)/2$.

are likely to become common. Such strategies are nice, provokable and forgiving, meaning that they are never the first to defect, punish a defection by another player by immediately defecting, and answer an act of cooperation by another player by immediately cooperating. Axelrod bases this conclusion on three findings: First, nice, provokable and forgiving strategies can increase in frequency in a population in which ALLD is common as long as there is some population structure. Second, computer tournaments indicate that once they get started, nice, provokable, forgiving strategies do extremely well against a wide variety of other strategies. Third, if a nice, provokable, forgiving strategy becomes common in a population where w is sufficiently high, Axelrod argues that it will remain common because such strategies are "collectively stable"⁹. A strategy S_e is collectively stable if for any possible strategy S_i :

$$V(S_e|S_e) \geq V(S_i|S_e) \quad (1)$$

where $V(S_m|S_n)$ means the expected fitness of individuals who use strategy S_m when interacting with individuals using strategy S_n . Axelrod argues that collective stability implies evolutionary stability, so that when a collectively stable strategy is common in a population and individuals are paired randomly, no other rare strategy can invade.

However, collective stability does not imply evolutionary stability. Suppose that a common strategy S_e coexists in a population with $N - 1$ rare strategies that are maintained in the population by mutation. Let the frequencies of the N strategies be $p_1, \dots, p_N$. To be evolutionarily stable, the common strategy, S_e , must have higher expected fitness than any rare invading strategy, S_i (ref. 1). Assuming that interacting individuals pair randomly, this requires:

$$\sum_{j=1}^N p_j V(S_e|S_j) > \sum_{j=1}^N p_j V(S_i|S_j) \quad (2)$$

If for each i , $V(S_e|S_e) > V(S_i|S_e)$, then S_e is both collectively and evolutionarily stable because $p_e \gg p_i$ ($e \neq j$). However, if equation (1) is satisfied for each i , but for some i , $V(S_e|S_e) = V(S_i|S_e)$, then S_e is collectively stable but may not be evolutionarily stable. This distinction is important in the repeated Prisoner's Dilemma game because there are many important pairs of strategies for which $V(S_e|S_e) = V(S_i|S_e)$ and $V(S_i|S_i) = V(S_e|S_i)$. For example, any nice strategy has the same expected fitness when interacting with any other nice strategy because neither ever defects. The relative fitness of such pairs of strategies depends on their interaction with other rare strategies. TFT is nice and (assuming w is sufficiently large) collectively stable¹⁰. Whether it can resist invasion by another nice strategy, for example TIT FOR TWO TATS (TF2T) which allows two consecutive defections before retaliating, depends on how TFT and TF2T do against rare non-nice strategies.

Using the criterion of evolutionary stability changes several of Axelrod's important results. First, when w is large enough, TFT can be invaded even though it is nice, provokable and forgiving. Assume that strategies are transmitted according to a haploid genetic model^{1,12,13} and that one-way mutation maintains SUSPICIOUS TIT FOR TAT (STFT), which defects on the first interaction and then plays TFT, in the population. Then TF2T can invade TFT whenever $V(\text{TF2T}|\text{STFT}) > V(\text{TFT}|\text{STFT})$. When w is large enough, this inequality is always

satisfied because the more tolerant TF2T induces STFT to cooperate, whereas the provokable TFT becomes involved in an endless sequence of reprisals. Second, strategies like TF2T which are not provokable can persist in a population at high frequency. If w is sufficiently large, the only population configuration in this system that is locally stable is a mixture of TF2T and STFT. The frequency of TF2T at this equilibrium, $\hat{p}$, is given by

$$\hat{p} \approx \frac{(1-w)S + wR - P}{(1-w)(T + S - R) + wR - P} \quad (3)$$

Thus as w increases, the equilibrium frequency of TF2T approaches one. For the parameter values used in Axelrod's tournaments, $\hat{p} = 0.9863$. Thus a population fixed for a nice, but intolerant morph (TIT FOR TAT) is replaced by a population which is a mixture of a nice, tolerant morph (TF2T) and a suspicious and somewhat exploitative morph (STFT). Finally, if w is large enough, ALLD can be invaded without population structure. For example, STFT can invade a population in which ALLD is common and in which TF2T is maintained by one-way mutation. Neither ALLD nor STFT are ever the first to cooperate; when paired they both defect during every interaction. Thus, their relative fitness depends on how each fares against TF2T. For w large enough, STFT has a higher expected payoff against TF2T than does ALLD, because STFT can be induced to cooperate whereas ALLD cannot. Thus, STFT can invade ALLD. The only stable equilibrium in this population is the mixture of TF2T and STFT given by equation (3). Although the results in this paragraph assume that rare variants are maintained by mutation, similar results obtain if rare variants are maintained by non-heritable phenotypic variation, or if they are due to repeated small perturbations in a model without mutation or phenotypic variation.

In fact, no strategy whose behaviour during interaction t is uniquely determined by the history of the game up to that point is evolutionarily stable if

$$w > \min[(T - R)/(T - P), (P - S)/(R - S)] \quad (4)$$

where $\min$ denotes the smaller of the two values in brackets. To prove this, let S_e be a collectively stable strategy and let S_1 be a distinct strategy which nonetheless behaves exactly the same way on each interaction with S_e as S_e does against itself. This implies that $V(S_1|S_e) = V(S_e|S_e) = V(S_e|S_1) = V(S_1|S_1)$. Thus, if a third strategy S_x exists in the population, S_e can be invaded by S_1 if $V(S_1|S_x) > V(S_e|S_x)$. Because S_e and S_1 are distinct, there must be some sequence of moves, A , during interactions $1, \dots, t-1$ such that S_e and S_1 react differently to S_x for the first time on move t . There are two possibilities. First, suppose S_e defects and S_1 cooperates on move t . Let S_2 be the strategy that generates the sequence A in response to both S_e and S_1 , cooperates on move t , and then defects forever in response to defection by S_e and cooperates forever in response to cooperation by S_1 on move t . S_e can be invaded by S_1 whenever:

$$V(S_1|S_2) - V(S_e|S_2) \geq w^{t-1}(R - T) + w^t(R - P)/(1 - w) > 0 \quad (5)$$

or $w > (T - R)/(T - P)$. Next, let S_3 be a strategy which behaves exactly like S_2 for the first $t-1$ moves, defects on move t , and then defects forever in response to S_e 's defection and cooperates forever in response to cooperation by S_1 on move t . In this case S_e can be invaded by S_1 whenever $w > (P - S)/(R - S)$. The second possibility is that S_e cooperates and that S_1 defects on move t . A similar argument shows that S_e can be invaded by S_1 for any value of w . This result can be understood in terms of Axelrod's insight that if w is large enough, no strategy can be best against all opponents. When two strategies interact with each other the same way that they do with themselves, their relative fitness depends on their interactions with other strategies. Because neither strategy can be best against every possible third strategy, no pure strategy can resist invasion by any combination of strategies.

This result does not mean that nice, provokable, forgiving strategies like TFT cannot persist in real populations. In reality, mutation and phenotypic variation will not generate every possible combination of invading strategies. Thus, whether nice, provokable and forgiving strategies can persist at high frequency in real populations will depend on the actual mix of rare non-nice strategies maintained in the population by mutation and phenotypic variation. For example, consider a population in which TFT is common, and STFT and ALLD are maintained by mutation. If the frequency of ALLD is low relative to STFT at mutation-selection equilibrium, TF2T can invade such a population. If, on the other hand, ALLD is relatively more common, TF2T cannot invade. Thus, TFT may or may not persist depending on the relative rates at which mutation creates the two non-nice variants. More generally our results suggest that the nature of the selective forces that shape ongoing, potentially altruistic social interactions depends on the distribution of rare variants that are created by mutation, environmental variation and other processes that maintain phenotypic variation. Although it seems likely that cooperative strategies will predominate when ongoing interactions persist over long periods of time, the nature of the contingent strategies that enforce this cooperation may depend on the kinds of non-adaptive variation that are present.

We thank David Ford for extensive discussion of this work.

Received 7 November 1986; accepted 23 February 1987.

1. Maynard Smith, J. *Evolution and the Theory of Games* (Cambridge University Press, 1982).
2. Brown, J. S., Sanderson, M. J. & Michod, R. E. *J. theor. Biol.* **99**, 319-339 (1982).
3. Aoki, K. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4065-4068 (1982).
4. Peck, J. R. & Feldman, M. W. *Am. Nat.* **127**, 209-221 (1985).
5. Bartholdi, J. J., Butler, C. A. & Trick, M. *J. Conflict Resolution* **30**, 129-140 (1986).
6. Axelrod, R. *J. Conflict Resolution* **24**, 3-25 (1980).
7. Axelrod, R. *J. Conflict Resolution* **24**, 379-403 (1980).
8. Axelrod, R. *Am. pol. Sci. Rev.* **75**, 306-318 (1981).
9. Axelrod, R. & Hamilton, W. D. *Science* **211**, 1390-1398 (1981).
10. Axelrod, R. *The Evolution of Cooperation* (Basic Books, New York, 1984).
11. Maynard Smith, J. *J. theor. Biol.* **47**, 209-221 (1974).
12. Taylor, P. D. & Jonker, L. B. *Math. Biosci.* **40**, 145-156 (1978).
13. Zeeman, E. C. *J. theor. Biol.* **88**, 249-270 (1982).

Adjuvant-free IgG responses induced with antigen coupled to antibodies against class II MHC

George Carayanniotis & Brian H. Barber*

Department of Immunology, Medical Sciences Building, University of Toronto, Toronto M5S 1A8, Canada

The generation of strong serological responses to protein antigens in experimental animals usually requires the use of potent adjuvants, most of which cannot be used in human or veterinary vaccines because of deleterious side effects^{1,2}. Attempting to circumvent this problem, we have assessed an adjuvant-free antigen-delivery system based on the hypothesis that antigen coupled to monoclonal antibodies (mAbs) specific for class II major histocompatibility complex (MHC) determinants should be 'targeted' onto antigen-presenting cells, thus facilitating recognition by helper T cells³. We found that the biotin-binding protein avidin⁴ could generate a serological response in mice, without adjuvant, when injected coupled to a biotinylated anti-class II MHC mAb. Equivalent amounts of avidin mixed with the non-biotinylated form of the same mAb failed to elicit a response. A targeting effect was demonstrated at low levels of injected conjugate because only mice bearing the appropriate class II antigens responded. Responses were also seen with a protein antigen other than avidin, offering a new, adjuvant-free approach to subunit vaccine construction.

* To whom correspondence should be addressed.

In mice, the serological response to avidin has been shown to be under genetic control, with $H-2^k$ being a low-responder, and $H-2^b$ a high-responder haplotype⁵. We have compared the anti-avidin response between (C57BL/6J (B6, $H-2^b$)) and (B6 $\times$ C3H)F₁ ($H-2^b \times H-2^k$) mice, because in this way we could target an (anti-I-A^k)-avidin conjugate onto F₁ cells without blocking the high-responder I-A^b determinants expressed on the same cell. The priming dose of avidin (5 or 50 μ g) was injected coupled to either biotinylated anti-I-A^k mAb (Fig. 1a) or biotinylated antibody to influenza virus nucleoprotein (NP) (control mAb, Fig. 1c). For comparison, equivalent amounts of avidin alone were injected in the presence or absence of Freund's complete adjuvant (Fig. 1d). In addition, mice were challenged with the same doses of avidin mixed with non-biotinylated anti-I-A^k mAb, as a control for possible immunoregulatory effects of the antibody itself⁶ (Fig. 1b). The sera obtained from these immunizations were tested for anti-avidin immunoglobulin G (IgG) responses following a secondary intraperitoneal (i.p.) boost with free avidin. The results can be summarized as follows. First, at the 5 μ g dose of avidin, a significant response was observed in (B6 $\times$ C3H) F₁ mice injected with the (anti-I-A^k)-avidin conjugate, whereas the B6 mice were not appreciably sensitized (Fig. 1a). This phenomenon could not be attributed to an immunostimulatory effect of the antibody alone, because the mixture of 5 μ g avidin with unmodified anti-I-A^k mAb did not elicit a response (Fig. 1b). An equal amount of avidin coupled to the anti-NP mAb also failed to generate an appreciable response (Fig. 1c), suggesting that the positive response in Fig. 1a can be attributed to more than the simple conjugation of avidin to an antibody. As expected, 5 μ g of avidin injected with Freund's complete adjuvant induced a strong serological response (Fig. 1d). Second, with the 50 μ g dose of avidin, free avidin plus anti-I-A^k mAb in the absence of adjuvant failed to stimulate a response (Fig. 1b), but in the form of (anti-I-A^k)-avidin complex, both the (B6 $\times$ C3H)F₁ and B6 mice responded (Fig. 1a). Responses in the B6 mice at the higher avidin dose may be attributed to a threshold effect resulting from increased uptake of conjugate by antigen-presenting cells. The (anti-NP)-avidin conjugate also appeared to be somewhat more immunogenic at the 50 μ g level (Fig. 1c). As expected, 50 μ g of free avidin in the presence (but not in the absence) of the adjuvant gave a strong serological response (Fig. 1d).

The results outlined above clearly indicate that low doses of avidin can be made immunogenic in the absence of adjuvant when presented to the immune system coupled to a mAb specific for recipient class II MHC determinants. This observation has been confirmed in four additional experiments and extended to the use of an anti-I-E^k as the targeting mAb. The (B6 $\times$ C3H)F₁ mice immunized with (anti-I-E^k)-avidin conjugate gave a 7–9-fold higher response than B6 mice that received the same dose of conjugate (Fig. 2a). Again unconjugated mixtures of avidin and mAb gave only background levels of reactivity (data not shown). Thus it appears that the enhancement of immunogenicity achieved by linking an antigen to an anti-class II mAb is not a unique property of a particular antibody, or in this case, of the specific class II determinant with which it reacts.

To determine whether or not conjugate uptake by Fc (crystallizable fragment) receptors is important in the enhancement of immunogenicity, we prepared (anti-I-E^k)-avidin conjugate using the F(ab')₂ (divalent antigen-binding fragment) of the mAb. This conjugate was injected into C3H and B6 mice to test at the same time whether enhancement of avidin immunogenicity could be seen in homozygous $H-2^k$ mice that lacked high responder $H-2^b$ -linked genes. The results (Fig. 2b) showed that two out of three C3H mice responded strongly to avidin. No detectable anti-avidin response was seen in the three B6 mice that were challenged. We conclude from these data that the Fc portion of the mAb is not essential for conjugate immunogenicity. The demonstration that the (anti-I-E^k)-avidin conjugate is immunogenic in homozygous $H-2^k$ mice indicates that the

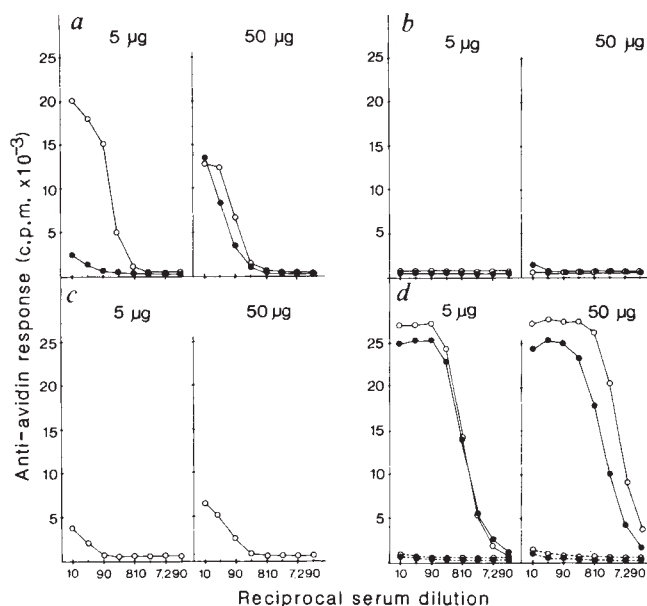


Fig. 1 The secondary anti-avidin response from (B6 $\times$ C3H)F₁ (○) or B6 (●) mice that were immunized with: (anti-I-A^k)-avidin conjugate (a), a mixture of non-biotinylated anti-I-A^k mAb and avidin (b), anti-influenza NP-avidin conjugate (c) and free avidin with (solid line) or without (broken line) Freund's complete adjuvant (d). All mice (the Jackson Laboratory, Bar Harbor; 6–12 weeks old) received the indicated equivalent amounts (left-hand graphs, 5 μ g; right-hand graphs, 50 μ g in each section) of avidin at a single site subcutaneously (s.c.) in 0.2 ml phosphate-buffered saline (PBS). After three weeks they were boosted intraperitoneally (i.p.) with 5 μ g free avidin in 0.2 ml PBS, and nine days later they were bled from the retro-bulbar sinus. Pooled sera from three mice were used in each group.

Methods. The hybridoma cell line TIB-92 (10–3.6.2 (ref. 13), anti-I-A^k) and HB-65 (H16-L10-4R5¹⁴, anti-influenza A NP) were obtained from the American Type Culture Collection and were cultured in RPMI 1640 supplemented with 10% fetal calf serum, L-glutamine and antibiotics. Both antibodies are of the IgG_{2A} subclass, and were purified from culture supernatants by protein A-Sepharose chromatography¹⁵. Biotinylation was performed according to the method of Goding¹⁶. The antibodies were dialysed against 0.1 M NaHCO₃, pH 8.2, and concentrated to 1.0 mg ml⁻¹. The biotin succinimide ester (Sigma) was dissolved in dimethylsulphoxide (DMSO) to 1.0 mg ml⁻¹ immediately before use and 60 μ l of ester added per ml of antibody at room temperature for 1 h. The biotinylated protein was dialysed overnight against PBS and stored at 4 °C. The biotinylated antibody-avidin conjugates were formed at a mixing ratio of 1 mol of egg avidin (Sigma) to 2 mol of antibody for 20 min at room temperature. The radioimmunoassay (RIA) used to monitor IgG responses was performed as follows. Avidin (1 μ g per 50 μ l per well) was incubated with polyvinyl chloride microtitre plates for 1 h, after which the plates were washed and the wells blocked with 0.1% BSA in PBS for an additional 1 h. The buffer was removed and 50 μ l of serial dilutions of immune sera in 0.1% BSA-PBS were added per well for 1 h. The plates were washed three times with 0.1% BSA-PBS and 50 μ l of ¹²⁵I-labelled protein A (Amersham) containing 65,000 c.p.m. was added for 1 h to each assay well. The plates were then washed twice and individual wells were cut for counting.

targeting antibody does not significantly interfere with the class II-restricted recognition processes required for antibody production.

Although there is no reason to suspect that avidin might represent a unique or special antigen in terms of testing the mAb-mediated delivery system, we have also tested whether this approach could be extended to different protein antigens, using bovine serum albumin (BSA) as a representative third molecule.

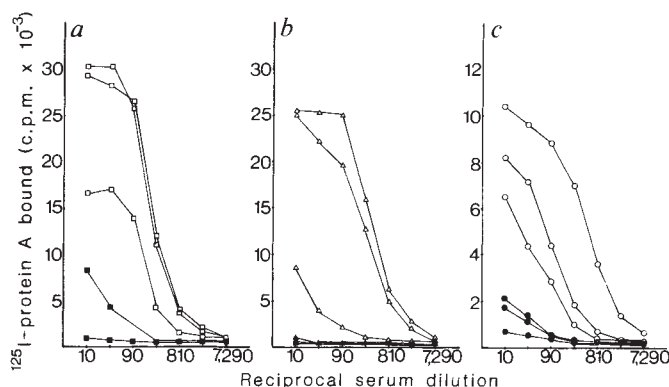


Fig. 2 Secondary responses to avidin and BSA. *a*, Secondary anti-avidin responses for individual (B6 × C3H) F_1 (□) or B6 (■) mice primed subcutaneously (s.c.) with ~25 µg avidin in the form of an (anti-I-E^k)-avidin conjugate. Three weeks after priming, all mice were boosted intraperitoneally (i.p.) with 10 µg free avidin in 0.2 ml PBS, and their sera were obtained nine days later. No response was observed in mice primed with an equivalent dose of avidin in an unconjugated mixture of non-biotinylated mAb and avidin (data not shown). The 14-4-4S antibody with anti-I-E^k specificity was purified from tissue culture supernatants of the hybridoma cell line HB-32 obtained from the American Type Culture Collection¹⁷. Conditions of culture, antibody purification and biotinylation are as described in Fig. 1 legend. Conjugates were formed by mixing 1 mol of avidin for every 2 mol antibody for 20 min at 20 °C followed by centrifugation for 5 min at 12,000g to remove any precipitable aggregates. Free biotin was also added in excess (30 mol biotin to 1 mol avidin) to block further interaction. *b*, Secondary anti-avidin responses for individual C3H (Δ) or B6 (▲) mice primed s.c. with ~25 µg avidin as an (anti-I-E^k)-avidin conjugate using the F(ab')₂ fragment of the mAb. A 6-h digestion of the intact antibody with pepsin was performed according to the method of Lamoyi and Nisonoff¹⁸ and cleavage confirmed by SDS-PAGE (polyacrylamide gel electrophoresis). The conditions of biotinylation and conjugation were as described in Figs 1 and 2a. After three weeks all mice were boosted i.p. with 10 µg of free avidin in 0.2 ml of PBS and their sera were obtained nine days later. *c*, Secondary anti-BSA responses for individual (B6 × C3H) F_1 (○) or B6 (●) mice primed s.c. with approx. 30 µg BSA in the form of an (anti-I-E^k)-avidin-BSA complex. This conjugate was formed by mixing equimolar amounts of biotinylated mAb, avidin and biotinylated BSA for 20 min at 20 °C, followed by centrifugation for 5 min at 12,000g to remove any precipitable aggregates. After three weeks mice were boosted i.p. with 10 µg of free BSA in PBS and their sera were obtained nine days later. The blocking buffer in the radioimmunoassay contained 0.1% ovalbumin in PBS and 65,000 c.p.m. of ¹²⁵I-protein A were added to each assay well.

Biotinylated BSA was conjugated to biotinylated anti-I-E^k mAb using avidin as a multivalent 'bridge'. The conjugates formed can successfully mediate a specific enhancement of BSA immunogenicity. Only (B6 × C3H) F_1 mice bearing the I-E^k antigens responded to BSA under this immunization protocol, whereas B6 mice which lack this allele remain essentially unresponsive (Fig. 2c). These data demonstrate the practicality of this method for generating serological responses to a variety of different protein antigens. More recent experiments using weekly multiple (3 times), rather than single, injections of the targeting complex have indicated that it is possible to reach levels of anti-avidin antibody responses comparable to those obtained by a single priming with Freund's complete adjuvant (data not shown).

The mechanism underlying the observed mAb-mediated increase in the immunogenicity of conjugated protein antigens is not known, but it seems reasonable to speculate that this effect is a direct consequence of bringing the complex into contact

with cells bearing class II MHC determinants. The proliferation of helper T cells recognizing processed antigen in the context of class II MHC products, and the subsequent generation of T-cell help, are considered essential requirements for the production of an effective secondary antibody response³. Under conditions where antigen is limiting, the antibody-mediated focusing of foreign determinants onto the surface of the antigen-presenting cell may promote immunogenicity by simply facilitating antigen uptake, resulting in a combination of processed antigen and class II products which efficiently promotes T-cell help⁷. Alternatively, it is possible that the targeting antibody may be involved more directly in the generation of T-cell help, such as by the induction of interleukin-1 secretion⁸. In addition, immunoglobulin-reactive helper T cells may promote the antigen-specific response through recognition of determinants on the processed targeting mAb^{9,10}.

Initial results from experiments using conjugates of avidin and anti-class I mAb (specific for H-2K^k) indicate that these complexes can also enhance the immunogenicity of avidin (data not shown). The level of response in C3H mice was similar to that depicted for the anti-class II complex in Fig. 2b. Thus in future work, it will be important to determine which cell-surface molecules represent target structures capable of mediating this enhancement of immunogenicity, and then define the events subsequent to binding which are relevant to more efficient activation of helper T cells.

Kawamura and Berzofsky¹¹ recently reported a successful attempt to target antigen to the immune system by coupling it to an antibody against immunoglobulin. They found that in the presence of Freund's incomplete adjuvant the immunogenicity of ferritin could be enhanced in mice by covalently coupling it to goat antibodies specific for mouse IgG. Both their data, and ours obtained in the absence of adjuvant, indicate that it is possible to augment the immunogenicity of protein antigens by using antibodies to focus foreign determinants onto specific cellular components of the immune system.

The demonstration that protein antigens coupled to anti-class II mAbs can elicit adjuvant-independent antibody responses offers a new approach to the establishment of protective immunity. As the ability to generate protein antigens by synthetic or gene cloning techniques increases, so does the possibility of constructing safe, effective subunit vaccines against a variety of different pathogens¹². Those antigens which are insufficiently immunogenic in the adjuvants currently available for human and veterinary use may possibly be made more effective using an appropriate mAb as a delivery agent.

We thank Eva Vizi for technical assistance. Support for this research was provided through the Program for Industry Laboratory Projects of the National Research Council of Canada and Connaught Laboratories Ltd.

Received 22 December 1986; accepted 3 March 1987.

- Warren, H. S., Vogel, F. R. & Chedid, L. A. *Rev. Immunol.* **4**, 369-388 (1986).
- Chapel, H. M. & August, P. J. *Clin. exp. Immunol.* **24**, 538-541 (1976).
- Schwartz, R. H. A. *Rev. Immunol.* **3**, 237-261 (1985).
- Green, N. M., *Adv. Protein Chem.* **29**, 85-133 (1975).
- Friedman, A. & Cohen, I. R. *Immunogenetics* **18**, 267-275 (1983).
- Borel, Y. *Immun. Rev.* **50**, 71-104 (1980).
- Matis, L. A., Glimcher, L. H., Paul, W. E. & Schwartz, R. H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6019-6023 (1983).
- Palacios, R. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6652-6656 (1985).
- Janeway, C. A. Jr in *Strategies of Immune Regulation* (eds Sercarz, E. & Cunningham, A. 179-198 (Academic, London, 1983).
- Leserman, L. *Immun. Today* **6**, 352-355 (1985).
- Kawamura, H. & Berzofsky, J. A. *J. Immunol.* **136**, 58-65 (1986).
- Liew, F. Y. *Clin. exp. Immunol.* **62**, 225-241 (1985).
- Oi, V. T., Jones, P. P., Goding, J. W., Herzenberg, L. A. & Herzenberg, L. A. *Curr. Topics Microbiol. Immunol.* **81**, 115-129 (1978).
- Yewdell, J. W., Frank, E. & Gerhard, W. *J. Immunol.* **126**, 1814-1819 (1981).
- Ey, P. L., Prowse, S. J. & Jenkin, C. R. *Immunochimistry* **15**, 429-436 (1978).
- Goding, J. W. *Monoclonal Antibodies: Principles and Practice*, 230-233 (Academic, London, 1983).
- Ozato, K., Mayer, N. & Sachs, D. *J. Immunol.* **124**, 533-540 (1980).
- Lamoyi, E. & Nisonoff, A. *J. Immun. Meth.* **56**, 235-243 (1983).

Cytotoxic T lymphocytes and glucocorticoids activate an endogenous suicide process in target cells

David S. Ucker*

Department of Pathology, Harvard Medical School, and Laboratory of Molecular Immunology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA

Cytotoxic T lymphocytes (CTL) induce a cytolytic process in target cells which, like the glucocorticoid-mediated cytotoxicity of immature thymocytes, effects a rapid and characteristic degradation of chromosomal DNA. I have explored the possibility that these two lethal processes share a common pathway by studying the susceptibility of glucocorticoid-resistant mutants to CTL-mediated killing. Here, I report that an unusual thymoma mutant, which has normal hormone receptor activity, is resistant to both glucocorticoids and CTL. The failure to be killed by CTL is not due to an inability of this 'deathless' mutant to be recognized. Further, a single-step reversion can restore sensitivity to both glucocorticoids and CTL. The genetic locus thus identified may reveal one element of an endogenous suicide pathway that can be triggered by different effectors. Unlike complement-mediated lysis, the processes of glucocorticoid- and CTL-mediated cytotoxicity seem to require that target cells be active in their own death.

The mechanism by which cells recognized as appropriate immune targets are killed by CTL is unresolved. One view is that targets are lysed in a manner analogous to the action of complement^{1,2}. For example, lesions observed on target cell membranes following lethal conjugation with cytotoxic cells^{3,4} could represent transmembrane channels formed by pore-forming proteins released by the granules of the cytotoxic lymphocytes⁵⁻¹³.

A different view of the CTL-mediated cytolytic process derives from the work of Russell and colleagues¹⁴⁻¹⁶. Within minutes of conjugation of CTL and targets, a degradative process is initiated in the target nucleus which ultimately results in quantitative conversion of the target genome into small DNA fragments¹⁵⁻¹⁷, integer multiples of a ~200-base-pair (bp) unit¹⁷.

Previous work with cortisone-sensitive thymocytes showed that treatment with glucocorticoid hormones results in an increased nuclear fragility¹⁸ and a similar pattern of DNA fragmentation^{19,20}. If CTL- and glucocorticoid-mediated cytolytic pathways share common elements, genetic defects which abolish cytotoxicity by glucocorticoids at steps distal to hormone receptor action might also confer resistance to killing by CTL.

DNA fragmentation can be observed following treatment of cells of the BALB/c (H-2^d)-derived glucocorticoid-sensitive thymoma, S49, with the synthetic glucocorticoid hormone, dexamethasone (Fig. 1a; also see ref. 21). When S49.A2.1 (a subclone of S49, referred to as A2.1) cells are cultured in 1 μ M dexamethasone for 10 h, ~30% of their DNA is converted to a 'ladder' of fragments of integer multiples of 180 bp (lane 3). By comparison, less than 2% of untreated control cell DNA exists as soluble fragments (lanes 1 and 2). A pattern of DNA fragmentation indistinguishable from that resulting from dexamethasone treatment can be seen in A2.1 cells following incubation with H-2^d-specific CTL (lane 4). Note, however, that CTL-induced degradation occurs much more rapidly than hormone-mediated fragmentation: ~30% of DNA is converted to fragments after only one hour of CTL treatment.

The induction of this DNA degradative process correlates with other measures of the cytolytic effect of glucocorticoids and CTL on A2.1 cells. Specifically, 1 μ M dexamethasone

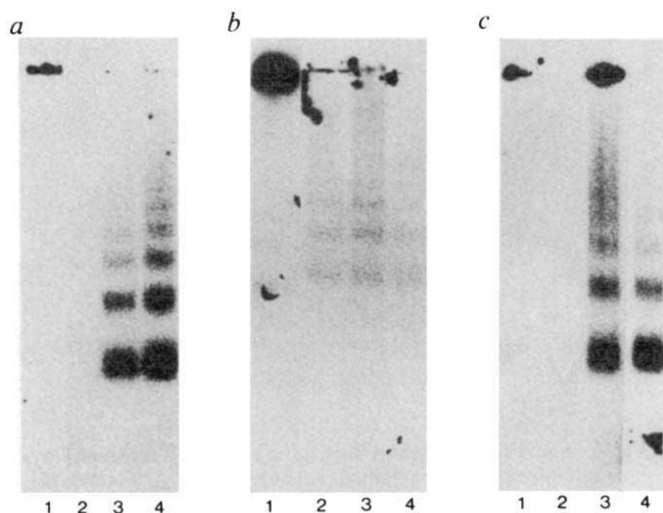


Fig. 1 Cellular DNA fragmentation after cytotoxic treatment. Wild-type A2.1 (a), deathless 4R.D (b) and revertant 4R.D.GCl (c) cells were labelled with ¹²⁵I-iododeoxyuridine. Cells (2×10^6) were incubated for 10 h in the absence (lanes 2) or presence (lanes 3) of 1 μ M dexamethasone, or with 1×10^7 H-2^d-specific CTL (responders from a 10-day primary unidirectional C57BL/6J anti-BALB/cBy mixed lymphocyte culture) for 1 h (lanes 4). Cells were disrupted in 0.2% Triton X-100 and 3 mM EDTA, and DNA from the supernatants recovered by precipitation¹⁶. In addition, labelled control cells (2×10^5) were lysed in 0.5% SDS and total DNA was precipitated (lanes 1). Samples were run on 2% agarose gels and autoradiographed. The extent of fragmentation was estimated by comparing ¹²⁵I-iododeoxyuridine in the Triton X-100 supernatant with the total input ¹²⁵I-iododeoxyuridine in each sample. For A2.1 and 4R.D.GCl cells (a and c, respectively), dexamethasone and CTL treated samples (lanes 3 and 4) correspond to ~30% fragmentation; less than 2% fragmentation was found in control samples (lanes 2). For 4R.D cells (panel b), all samples (lanes 2, 3 and 4) show ~2% fragmentation. Comparison of the labelled bands with *Hae*III-digested ϕ X174 DNA size markers indicates that the ladder of fragments corresponds to integer (n) multiples of a 180-bp unit. The lowest band observed in each panel of lanes represents fragments 360 bp (n = 2) in length; 180-bp (n = 1) fragments are not resolved in these gels.

Methods. Six spontaneous glucocorticoid-sensitive revertants of the deathless 4R.D mutant were recovered from a freshly subcloned culture of 4R.D (initially 5×10^6 cells) after two rounds of counter-selection against cells which exhibited the mutant phenotype and continued to grow after the addition of 1 μ M dexamethasone²⁶. In principle, proliferating cells are killed by incorporation of cytosine arabinoside; hormone-sensitive revertants that are growth-inhibited by dexamethasone (but have not yet died) are spared and enriched. After 8 h in dexamethasone and 5 μ g ml⁻¹ cytosine arabinoside, cells were washed repeatedly and resuspended in normal medium. One week after the second round of treatment, cells were plated at limiting dilution in microwells. Colonies which were visible after another three weeks were tested for sensitivity to 1 μ M dexamethasone.

dramatically inhibits the rate of growth of these cells and abolishes their ability to form colonies in microwells (plating efficiency is reduced to less than 0.1% compared with 73% in the absence of hormone). A four-hour incubation with H-2^d-specific CTL similarly lowers the efficiency of plating of A2.1 to only 6% and effects the efficient release of radiolabelled (⁵¹Cr)-chromium from preloaded A2.1 cells (Fig. 2a).

The glucocorticoid-resistant S49 mutant 4R.D, a subclone of the S49.61.4R 'deathless' isolate²², was selected for its ability to clone in medium containing 1 μ M dexamethasone and is unusual among glucocorticoid-resistant clones in retaining apparently wild-type glucocorticoid receptor^{22,23}. Remarkably, CTL have no cytolytic effect on 4R.D cells. The same H-2^d-specific CTL which reduced the efficiency of plating of parent-

* Present address: Medical Biology Institute, Division of Immunology, 11077 North Torrey Pines Road, La Jolla, California 92037, USA.

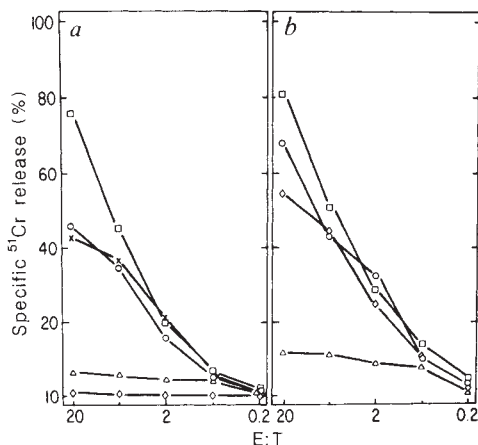


Fig. 2 CTL-induced chromium release from target cells; ○, wild-type A2.1; △, deathless 4R.D.; ×, revertant 4R.D.GC1; □, P815; ◇, EL4. Cells were labelled with sodium (^{51}Cr)-chromate and tested for susceptibility to lysis by H-2^d-specific CTL in *a*, the absence and *b*, the presence of $5\text{ }\mu\text{g ml}^{-1}$ concanavalin A. P815 and EL4 cells (H-2^d and H-2^b, respectively) serve as control targets. Reactions (200 μl volume) were performed in V-bottom 96-well plates, using 1×10^4 ^{51}Cr -chromium-labelled targets and effector CTL at the indicated effector to target (E:T) cell ratios, and incubated for 4 h at 37°C . Plates were centrifuged briefly and 100 μl of each supernatant was collected for determination of (^{51}Cr)chromium released. Specific chromium release was calculated according to the formula: (experimental release - spontaneous release) $\times 100$ / (maximal release - spontaneous release). Spontaneous release was ~20% for the S49-derived cells (A2.1, 4R.D. and 4R.D.GC1) and less than 5% for P815 and EL4. Maximal release was determined by lysing targets with 0.25% SDS.

like A2.1 cells by more than 10-fold had virtually no effect on the cloning efficiency of the deathless mutant (56% following CTL treatment versus 61% in control experiments). In a standard chromium-release assay, 4R.D. cells also appear refractory to the action of CTL (Fig. 2*a*). Again, a correlation between cytolysis and DNA fragmentation can be made. In resistant 4R.D. cells, fragmentation is not induced by glucocorticoids or by CTL (Fig. 1*b*). (Because 4R.D. cells consistently label with ^{125}I -iododeoxyuridine 5- to 7-fold better than A2.1 cells, it is easier to observe the background of fragmentation in the mutant.)

The resistance of 4R.D. cells to CTL is not a failure of recognition. The level of expression of H-2 determinants on the surface of 4R.D. cells, as seen cytofluorimetrically, is indistinguishable from parent-like A2.1 cells (data not shown). Similar results are obtained by comparing the susceptibility of A2.1 cells and 4R.D. cells to treatment with complement plus antibodies that react with specific H-2^d products (Fig. 3).

Significantly, these complement experiments demonstrate that the 4R.D. deathless mutant is as susceptible to the membrane damage and subsequent lysis caused by complement as is the wild-type A2.1 cell. The defect that renders 4R.D. cells insensitive to the cytolytic action of CTL and glucocorticoids does not confer resistance to the action of complement.

More directly, no significant differences exist in the frequencies with which H-2^d-specific CTL can form conjugates with CTL-resistant 4R.D. cells and parent-like A2.1 cells (not shown). Further proof that the deathless mutant is as capable of being recognized by CTL as is the parent-like A2.1 cell type is presented in Fig. 4. The addition of excess unlabelled A2.1 cells into a standard chromium-release reaction of H-2^d-specific CTL and (^{51}Cr)-chromate loaded A2.1 targets results in competitive displacement of the labelled cells. Deathless 4R.D. cells compete for CTL binding with wild-type A2.1 cells as well as A2.1 cells themselves. Thus, the 4R.D. cells, although refractory to the

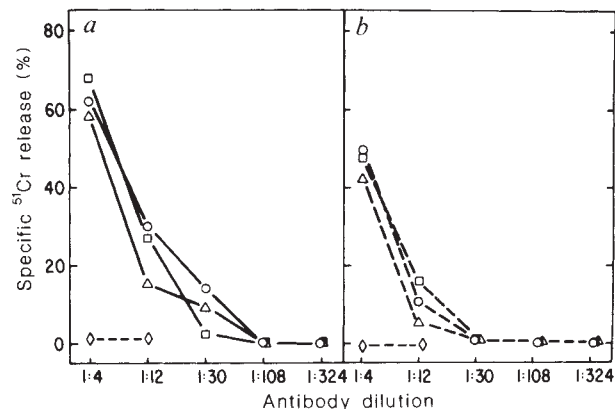


Fig. 3 Antibody plus complement-induced chromium release from target cells. (^{51}Cr)-chromium release assays were performed and analysed as in Fig. 2, except that each well contained 1×10^5 labelled target cells and no effector CTL. Cells and the indicated dilution of antibodies (as hybridoma culture supernatant) were incubated at 4°C for 60 min in 180 μl reactions. Complement (20 μl per well) was added and plates were shifted to 37°C for 30 min before supernatants were collected and analysed. Spontaneous release here was determined by treating cells with complement in the absence of antibody. *a*, 34-7-23S anti-(H-2K^d and H-2D^d) antibody²⁷; *b*, 34-4-21S anti-H-2D^d antibody²⁷. Symbols as in Fig. 2.

cytolytic consequence of CTL recognition, are unimpaired in their ability to be recognized.

Finally, chromium-release experiments were performed in the presence of the lectin concanavalin A (Fig. 2*b*). Even in these experiments, where specific recognition is not required, the deathless 4R.D. mutant is not susceptible to CTL-mediated killing.

The nature of the defect which gave rise to the deathless mutant is unknown. If a single lesion is responsible for resistance both to glucocorticoids and to CTL, a reversion event which re-establishes wild-type sensitivity to one cytolytic agent would be expected to restore sensitivity to the other. Spontaneous glucocorticoid-sensitive revertants of 4R.D. were recovered at a frequency of at least 2×10^{-7} (see Fig. 1). One of the revertant isolates, 4R.D.GC1, was further characterized.

As with the A2.1 wild-type line, the ability of the 4R.D.GC1 revertant to form colonies in microwells is lost in the presence of $1\text{ }\mu\text{M}$ dexamethasone (plating efficiency reduced from 43% to less than 0.1%) and DNA fragmentation, once again, is induced by the hormone (Fig. 1*c*). The 4R.D.GC1 revertant also appears indistinguishable from the parent-like A2.1 line as a target for CTL-mediated cytolysis. The extents of chromium release (Fig. 2*a*), colony-forming capacity at limiting dilution (3%) and nuclear DNA fragmentation (Fig. 1*c*) effected by CTL are equivalent to the A2.1 wild-type in 4R.D.GC1 cells.

Thus a single, spontaneous reversion event, recovered in the 4R.D.GC1 clone was able to restore the 4R.D. deathless mutant to the wild-type phenotype. A single genetic lesion, then, is responsible for the deathless phenotype; the normal gene activity of that locus in a target cell must be required for that susceptible target to respond—by dying—to the cytolytic signal provided either by CTL or by glucocorticoids. These data suggest that target cells are active in their own programmed death. This is clearly the case for the glucocorticoid-mediated killing of thymocytes: hormone-induced death of transformed thymoma cells occurs at limiting dilution in the absence of other cell types and must be cell-autonomous. But the implications of these results with respect to the target cytolysis mediated by CTL are provocative. Deathless target cell mutants would not be expected if the pore-forming activity of cytolytic granules recovered from CTL

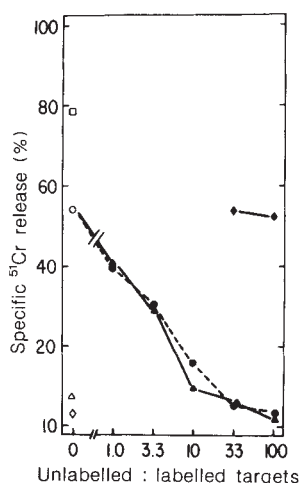


Fig. 4 Cold-target inhibition of CTL-induced chromium release. CTL-mediated (^{51}Cr)-chromium release was determined as in Fig. 2. Each well contained 1×10^4 (^{51}Cr)-chromium labelled targets (open symbols, as in Fig. 2) and 2×10^5 H-2^d-specific effector CTL (the effector to labelled target cell ratio was 20:1). In addition, unlabelled target cells (closed symbols) were included with labelled A2.1 cells at the indicated ratios.

induces colloid osmotic lysis of the target, as has been proposed²⁴. CTL, then, must act by inducing an autolytic process²⁵ in their targets. Glucocorticoids also must activate such a lytic programme in susceptible cells. The genetic lesion responsible for the deathless phenotype identifies one locus of what must be a common endogenous suicide pathway.

I thank Keith Yamamoto and Carol Sibley for S49.61.4R cells, Hildegund Ertl for advice about mixed lymphocyte cultures, Abul Abbas and Ken Rock for antibodies, Carolyn Pettey for help with conjugate assays, Mike Stallcup for encouragement and the cytosine arabinoside protocol, and Debby Dobson, Herman Eisen, Laurie Glimcher, Hans Oettgen, Carolyn Pettey, Peter Reczek, Ken Rock, Mike Stallcup and Tom Wileman for constructive comments. Finally, I thank Cox Terhorst for allowing me to work in his laboratory. This work was begun when I was a fellow of the Helen Hay Whitney Foundation and is now supported by the NIH.

Received 28 August 1986; accepted 16 February 1987.

- Podack, E. R. & Tschopp, J. *J. biol. Chem.* **257**, 15204-15212 (1982).
- Tschopp, J., Müller-Eberhard, H. J. & Podack, E. R. *Nature* **298**, 534-538 (1982).
- Dourmashkin, R. R., Deteix, P., Simone, C. B. & Henkart, P. *Clin. exp. Immun.* **42**, 554-560 (1980).
- Dennert, G. & Podack, E. R. *J. exp. Med.* **157**, 1483-1495 (1983).
- Henney, C. S. *J. Immun.* **110**, 73-84 (1973).
- Martiz, E., Burakoff, S. J. & Benacerraf, B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 177-181 (1974).
- Henkart, M. P. & Henkart, P. A. *Adv. exp. Med. Biol.* **146**, 227-242 (1982).
- Podack, E. R. & Dennert, G. *Nature* **302**, 442-445 (1983).
- Henkart, P. A., Millard, P. J., Reynolds, C. W. & Henkart, M. P. *J. exp. Med.* **160**, 75-93 (1984).
- Podack, E. R. & Konigsberg, P. J. *J. exp. Med.* **160**, 695-710 (1984).
- Masson, D. & Tschopp, J. *J. biol. Chem.* **260**, 9096-9072 (1985).
- Young, J. D.-E., Hengartner, H., Podack, E. R. & Cohn, Z. A. *Cell* **44**, 849-859 (1986).
- Young, J. D.-E., Nathan, C. F., Podack, E. R., Palladino, M. A. & Cohn, Z. A. *Proc. natn. Acad. Sci. U.S.A.* **83**, 150-154 (1986).
- Russell, J. H., Masakowski, V. R. & Dobos, C. B. *J. Immun.* **124**, 1100-1105 (1980).
- Russell, J. H. & Dobos, C. B. *J. Immun.* **125**, 1256-1261 (1980).
- Russell, J. H., Masakowski, V., Rucinsky, T. & Phillips, G. *J. Immun.* **128**, 2087-2094 (1982).
- Duke, R. C., Chervenak, R. & Cohen, J. J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6361-6365 (1983).
- Nicholson, M. L. & Young, D. A. *Cancer Res.* **38**, 3673-3680 (1978).
- Wyllie, A. H. *Nature* **284**, 555-556 (1980).
- Cohen, J. J. & Duke, R. C. *J. Immun.* **132**, 38-42 (1984).
- Vedeckis, W. V. & Bradshaw, H. D. *Jr Molec. Cell. Endocr.* **30**, 215-227 (1983).
- Sibley, C. H. & Tomkins, G. M. *Cell* **2**, 221-227 (1974).
- Yamamoto, K. R., Gehring, U., Stampfer, M. R. & Sibley, C. H. *Recent Progr. Horm. Res.* **32**, 3-32 (1976).
- Podack, E. R. *Immun. Today* **6**, 21-27 (1985).
- Russell, J. H. *Immun. Rev.* **72**, 97-118 (1983).
- Rabinbran, S. K., Danielsen, M., Firestone, G. L. & Stallcup, M. R. *Somat. Cell molec. Genet.* **13**, 131-143 (1987).
- Ozato, K., Mayer, N. M. & Sachs, D. H. *Transplantation* **34**, 113-120 (1982).

Expression and functional characterization of artificial mutants of interleukin-2 receptor

Shigeru Kondo, Masahiko Kinoshita*, Akira Shimizu*, Yuji Saito, Mikio Konishi, Hisataka Sabe & Tasuku Honjo

Department of Medical Chemistry, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606, Japan

The physiological proliferation of T lymphocytes (T cells) requires interaction between the humoral growth factor, interleukin 2 (IL-2) and its cell-surface receptor^{1,2}. Studies of IL-2 binding to the IL-2 receptor (IL-2R) on T cells have revealed that there are two distinct species of IL-2R, one with high and one with low affinity³. Isolation and characterization of cDNA for the human IL-2R⁴⁻⁶ made it possible to deduce the complete primary sequence (251 residues) of the receptor protein. However, expression of IL-2R alone is not sufficient for either growth signal transduction or high-affinity site formation^{7,8}; another lymphocyte-specific molecule called converter seems to be required for the biological activity of IL-2R⁹⁻¹³. We found that the converter did not form a stable complex with IL-2R unless the receptor bound the ligand (the 'affinity conversion' model)^{12,13}. To discover which are the functionally important parts of the human IL-2R we have constructed artificial mutant cDNAs encoding the receptor. The mutant receptors produced from them had deletions or substitutions in the cytoplasmic region (13 residues), the transmembrane region (19 residues) or the carboxy-terminal portion of the extracellular region (219 residues). All were active in growth signal transduction, efficient internalization and high-affinity site formation in two mouse T-cell lines, suggesting that the extracellular region of IL-2R and the converter may be responsible for growth signal transduction.

We have created three different types of mutant complementary DNAs for human IL-2R as shown in Fig. 1. The first group includes three mutants which contain substitutions and deletions in the transmembrane and cytoplasmic regions. In the 'P' mutant, glycine has replaced serine 247, which is the major target of phosphorylation^{14,15}. The 'Term' mutant, with only five amino-acid residues in the cytoplasmic region was made by inserting the termination codon TGA at position 243. In the 'Mem' mutant two serine residues (231 and 236), which are polar yet conserved in the hydrophobic transmembrane regions of mouse and human, are replaced with hydrophobic isoleucine residues. The second group are called 'Long-neck' and mutants Long-neck 6, 12 and 36 have insertions of 6, 12 or 36 amino-acid residues, respectively, between residues 211 and 212, eight residues on the amino-terminal side of the transmembrane region. The Long-neck mutants were constructed to test whether the distance between the IL-2-binding domain and the membrane is important. The third group consists of a mutant I-S-E, in which the transmembrane region and the amino-terminal 71 residues of the cytoplasmic region of the epidermal growth factor receptor (EGFR) were attached to the extracytoplasmic region of IL-2R at residue 212. The short sequence derived from EGFR does not contain the tyrosine kinase domain. The structures of all the mutant cDNAs were confirmed by nucleotide sequence determination.

All the mutant cDNAs were cloned in the pKCR.H2 vector⁴ (Fig. 1) and each cDNA was introduced into mouse T cells; line CTLL-2 which expresses the native murine IL-2R and EL-4, which expresses no IL-2R. Several clones of CTLL-2 and EL-4 expressing a large number ($>10^4$) of human receptors were isolated for each receptor mutant. Because the sizes of the mutant

* Present addresses: Research and Development Division, Rohto Pharmaceutical Co. Ltd, Osaka, Japan (M.K.); Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA (A.S.).

Table 1 Properties of IL-2 receptor mutants expressed on CTLL cells

Clone	cDNA	Thymidine incorporation (%)			Human IL-2R			
		Antibodies added			High-affinity		Low-affinity	
		PC61	anti-Tac	PC61 and anti-Tac	Sites per cell $\times 10^{-2}$	K_d (pM)	sites per cell $\times 10^{-4}$	K_d (nM)
CT/hR-1	Wild type	104	101	11	30	31	19	8.8
CT/P ⁻ -9	P ⁻	83	105	7	15	73	12	7.1
CT/Term-1	Term	86	104	4	22	66	4.7	9.4
CT/Mem-1	Mem	84	100	1	25	166	13	10.4
CT/LN6-1	Long-neck 6	86	131	15	30	50	12	12.8
CT/LN12-8	Long-neck 12	57	98	13	ND		ND	
CT/LN36-2	Long-neck 36	76	116	12	<0.5		9	7.2
CT/LN36-3	Long-neck 36	60	100	3	18	40	16	11.7
CT/I-sE-1	I-sE	60	98	10	<0.5		1.5	3.0
CTLL-2	—	2	99	4	—		—	

Transfection of CTLL-2 and selection of clones were done as described⁹. IL-2-dependent thymidine uptake of mutant CTLL clones was measured in the presence of anti-murine IL-2R antibody PC61¹⁶, anti-human IL-2R antibody anti-Tac¹⁷, or both. Assay of ³H-thymidine incorporation was as described⁹. Values are means of two or three experiments. Background incorporation without IL-2 was subtracted from the data obtained, and responses are expressed as the percentages of ³H-thymidine uptake relative to the value for each clone without antibody. The IL-2 dependence was strictly retained for all the mutant clones. The IL-2 concentration which gives half maximal incorporation of ³H-thymidine into each clone in the absence of antibodies was almost equal to that of CTLL-2 except for CT/LN36-2 and CT/I-sE-1. Maximal thymidine incorporation into the mutant clones in the presence of an excess of IL-2 was almost equal to that into CTLL-2 (data not shown). Though CT/LN36-2 and CT/I-sE-1 responded to IL-2 in the presence of PC61, they required three to five times higher concentrations of IL-2 for the half maximal response than the other clones. We isolated only one clone of the I-sE mutant expressing more than 10⁴ sites per cell. Other CT/I-sE clones expressing smaller numbers (>10³) of mutant receptors did not show clear growth stimulation by IL-2. The concentrations of human recombinant IL-2 used were 7.5 $\times 10^{-5}$ μ g ml⁻¹ for CT/LN36-2 and CT/I-sE-1; 2.5 $\times 10^{-5}$ μ g ml⁻¹ for the other clones. Final concentrations of PC61 and anti-Tac antibodies were 40-fold dilution of ascites fluid and 25 μ g ml⁻¹, respectively.

* Calculated from data of Fig. 2. ND, not determined.

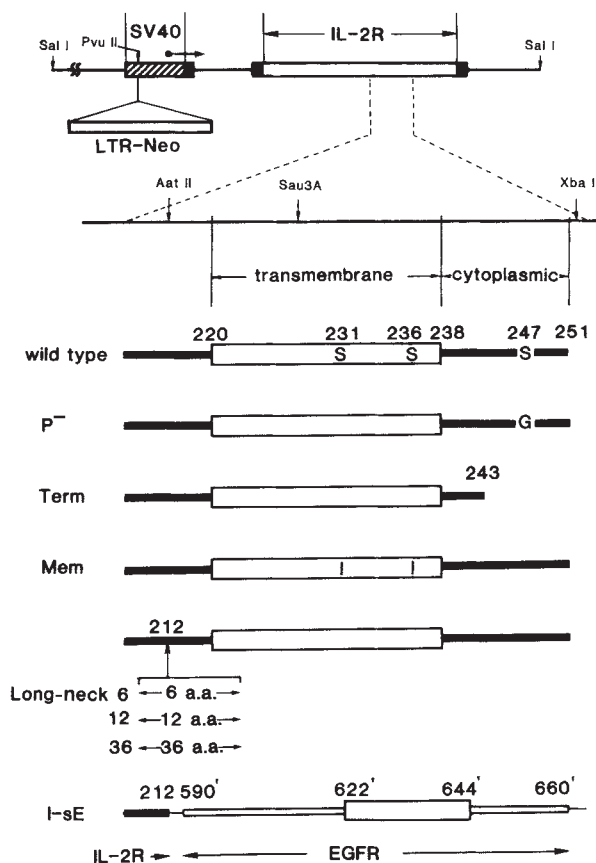


Fig. 1 Structures of mutant cDNAs for IL-2R. Above, structure of the expression vector pKCR-Tac2A⁴ and the restriction enzyme sites used for mutant plasmid construction; below, mutated regions enlarged. Open rectangles, transmembrane regions; amino-acid residues numbered from the N-terminus of the mature receptor. Amino-acid numbers of native EGFR (EGFR)²⁰ are shown with a prime: the transmembrane domain of the EGFR is from 622' to 644'. Vertical arrow at position 212, site of insertion; a.a., amino acid; S, serine; G, glycine; I, isoleucine.

Methods. Plasmids were constructed from pKCR-Tac2A as follows. For P⁻, the codon for serine 247 (AGT) was changed to the GGT for glycine. (Details have been described elsewhere^{7,21}.) For Term and Mem, the DNA fragment between the *Sau3A* and *XbaI* sites (66 base pairs (bp)) was replaced by mutated sequences. The exchanged codon of Term was CAG (Glu) $\rightarrow$ TAG (termination) at position 247, and those of Mem were AGC (Ser) $\rightarrow$ ATC (Ile) at position 231 and AGT (Ser) $\rightarrow$ ATT (Ile) at position 236. For Long-neck 6, 12, and 36, a 18-bp sequence (CA ATC TTC ATG GAA ACG T), a 36-bp sequence (CA ATC TTC ACG GAG ATG GCC GCG ACA ATG GAA ACG T) or three tandem 36-bp sequences, respectively, were inserted at the *AatII* site. The inserts were designed so that the amino-acid sequence Glu-Met-Ala-Ala-Thr-Met-Glu-Thr-Ser-Ile-Phe-Thr between positions 204 and 216 became duplicated. Codon usages of this inserted sequence were changed from that of the wild type to avoid recombination between insert and native sequences. For I-sE, the *AatII*-*XbaI* fragment (122 bp) was replaced by the *NaeI*-*PstI* fragment of the EGFR cDNA²¹ using synthetic linker DNA. The 5' and 3' sequences derived from the linker DNA were GTA CCC (Val-Pro) and GTC GAC TCT AGC (Val-Asp-Ser-Ser), respectively. The 2.5-kilobase (kb) LTR-Neo^r fragment (*Clal*-*StuI*) of Zip-Neo SV(X)1 plasmid²² was inserted in the *PvuII* site of each mutant plasmid.

proteins are not very different from that of the wild-type receptor, we could not confirm the structures of the mutants at the protein level. The clones examined and their activities are summarized in Tables 1 and 2.

As shown previously⁹, the human IL-2R expressed on CTLL-2 (clone CT/hR-1) is capable of transducing the growth signal in

response to IL-2. Because human recombinant IL-2 can react with the endogenous murine receptor, stimulation of thymidine incorporation by human IL-2 was measured in the presence of either antibody to murine IL-2R antibody (PC61)¹⁶, antibody to human IL-2R (anti-Tac)¹⁷ or both. Surprisingly, all the clones expressing the human mutant receptors responded to human IL-2 as measured by stimulation of thymidine incorporation in the presence of PC61 (Table 1). The presence of both PC61 and

Table 2 Properties of IL-2 receptor mutants expressed on EL-4 cells

Clone	cDNA	Human IL-2R				Internalization	Growth inhibition
		High-affinity Sites per cell $\times 10^{-2}$	K_d (pM)	Low-affinity Sites per cell $\times 10^{-4}$	K_d (nM)		
EL/Tac 3	wild type	12	12	6	12	28.1	4.5
EL/P ⁻ -2	P ⁻	14	9	20	10	25.6	2.5
EL/Term-3	Term	3	15	5	10	40.5	2.3
EL/Mem-7	Mem	46	16	38	7	33.2	4.0
EL/LN6-1	Long-neck 6	<0.5		11	11	3.3	0.95
EL/LN12-10	Long-neck 12	<0.5		7	7	1.0	1.1
EL/LN12-11	Long-neck 12	30	17	20	11	17.8	3.2
EL/LN36-1	Long-neck 36	<0.5		6	10	2.5	1.2
EL/I-sE-7	I-sE	<0.5		6	9	4.6	1.1
EL/I-sE-11	I-sE	20	25	11	9	21.0	4.0
EL-4	none	0		0		0	1.07

Transfection of EL-4 and selection of clones¹⁰ and the binding assay of ¹²⁵I-labelled IL-2⁷ were as described. Numbers (K_d) of high- or low-affinity sites and their dissociation constants were calculated from Scatchard plots. Internalization of the ligand-bound IL-2R is expressed as the ratio of internalized IL-2 to the total IL-2 bound at 15 min. Cells (2×10^6) were incubated in 100 μ l growth medium (RPMI 1640, 10% fetal calf serum (FCS), 50 μ M 2-mercaptoethanol) containing 0.4 ng ml⁻¹ ¹²⁵I-labelled IL-2 at 0 °C for 1 h, then incubated at 37 °C. After 15 min, 1 ml of 0.2 M glycine-HCl (pH 2.8) was added and the mixture was kept on ice for 5 min. Cells were separated from the medium by centrifugation through an olive-oil cushion and radioactivities associated with cells were counted with a gamma counter. Total bound radioactivity was counted in the same way, but without a glycine-HCl wash. Nonspecific binding was determined in parallel in the presence of 10 μ g ml⁻¹ of non-labelled IL-2. Growth inhibition¹⁰ is expressed as the ratio of cell numbers in the absence to those in the presence of 250 ng ml⁻¹ of recombinant human IL-2 after 72 h incubation. The concentration of cells at zero time was adjusted to 10⁴ ml⁻¹. Cells were counted under the microscope.

anti-Tac antibodies abolished the growth stimulation completely. It is therefore clear that all the mutant receptors are active in growth-signal transduction. We have assayed two or three clones of each mutant except for I-sE and have confirmed that all the mutant clones were active in stimulating growth. These results suggest that only the extracellular domain of IL-2R might be necessary for growth-signal transduction.

To determine whether the mutant human receptors can form high-affinity binding sites, Scatchard plot analysis was done for each clone using ¹²⁵I-labelled IL-2. CTLL clones expressing the P⁻, Term, Mem and Long-neck 6 mutant receptors, or the wild-type receptor (CT/hR-1) contained human high-affinity sites (Fig. 2a-e). The experiments were done in the presence of another antibody to mouse IL-2R (3C7)¹⁸, which also blocks IL-2 binding to native murine receptor of CTLL-2 (Fig. 2j).

As EL-4 has no endogenous murine IL-2R¹⁰, IL-2 binding and subsequent receptor internalization could be measured without the monoclonal antibodies. In agreement with the results from the CTLL clones, all the mutant receptors except for Long-neck 6 and Long-neck 36 expressed on EL-4 contained high-affinity sites, although the numbers were variable (Table 2).

Although numbers of high- and low-affinity sites varied, even among different clones of the same mutant receptor, these mutations did not seem to damage the ability of IL-2R to form high-affinity sites. In some clones of Long-neck and I-sE mutants the number of high-affinity sites was very small. For example, the Long-neck 36 mutant receptor on CT/LN36-2 gave rise to sites that were almost all of low affinity whereas CT/LN36-3 had a usual number of high-affinity sites (Fig. 2f and g). The difference is certainly not due to the cDNA construct but probably to the variable number of converter molecules expressed in each clone, because CT/LN36-2 and CT/I-sE-1 had few high-affinity sites of either human or mouse receptor (Fig. 2f and h). The results are consistent with the hypothesis that the secondary protein converter is required for high-affinity site formation and that the number of converters expressed in each clone is variable. Therefore, we conclude that all the mutant receptors constructed can form high-affinity binding sites and that only the extra-cellular domain of IL-2R is necessary for this activity.

The time course of internalization of bound IL-2 by each EL-4 clone was measured (Table 2). We found that ¹²⁵I-labelled IL-2 was rapidly internalized by the clones expressing a sig-

nificant number (>50) of high-affinity sites of all the mutant receptors. By contrast, the clones (EL/LN6-1, EL/LN12-10, EL/LN36-1 and EL/I-sE-7) that did not contain a significant number of high-affinity species of the IL-2R were unable to internalize IL-2 rapidly, in agreement with previous studies^{7,8}.

Growth inhibition by IL-2 was taken as evidence of signal transduction by IL-2R in EL-4 cells which grow without the growth factor¹⁰. All the clones which expressed a significant number of high-affinity species of mutant receptors showed growth inhibition by IL-2. The EL-4 clones (EL/LN6-1, EL/LN12-10, EL/LN36-1 and EL/I-sE-7), which did not contain a significant number of high-affinity species, did not respond to IL-2 although the same type of CTLL clones (CT/LN36-2 and CT/I-sE-1) did so at higher concentrations. The difference may be ascribed to the different nature of the parental cells and the different assays used. Alternatively, such EL-4 clones may have absolutely no high-affinity IL-2R whereas the corresponding CTLL clones may have some. Studies of the EL-4 clones of the IL-2R mutants clearly indicate that the expression of the high-affinity species of all the IL-2R mutants is associated with efficient internalization and growth inhibition by IL-2.

Functional studies on the artificial mutants of IL-2R described above have shown that conservation of the primary structures of the C-terminal portion of the extracellular region, the transmembrane region and the cytoplasmic region is not necessary for the formation of the high-affinity receptor species, growth-signal transduction or efficient internalization. Accumulating evidence indicates that the secondary molecule converter is required for growth signal transduction from IL-2R into the cytoplasm⁷⁻¹³. If so, the interaction between IL-2R and the converter must occur outside the cell directly or indirectly. This conclusion gained support from the recent report¹⁹ that HIEI, an antibody to human IL-2R, blocks formation of high-affinity sites without reducing the number of low-affinity sites. Because the Long-neck mutants were also active, the interaction between IL-2R and the converter may not take place immediately adjacent to the membrane. The elongated region may be flexible enough to allow interaction of the extracytoplasmic region and the converter at variable distance. This implies that a significant portion of the converter may be outside the membrane.

It is likely that the transmembrane and cytoplasmic regions are not directly involved in growth-signal transduction. However, extensive conservation of these regions in the human

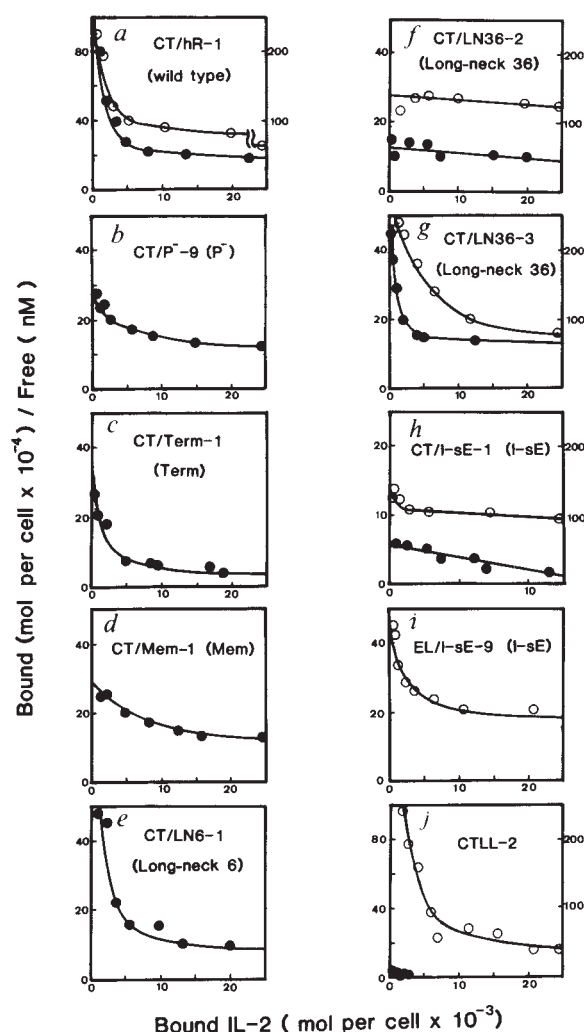


Fig. 2 A Scatchard plot analysis of IL-2R mutants. Equilibrium binding of ^{125}I -labelled IL-2 to the mutant receptors on CTLL-2 was carried out in the presence (●) or absence (○) of 3C7¹⁸, an antibody to murine IL-2R. The vertical scales on the left and right axes are for the plots with and without 3C7, respectively. The ^{125}I -labelled recombinant human IL-2 (Amersham) had specific activity 110,000 c.p.m. ng^{-1} . Binding of ^{125}I -labelled IL-2 was assayed as described⁹ except that cells were incubated in RPMI 1640 containing 40% 3C7 ascites fluid for 30 min at 0 °C before IL-2 binding. The final concentration of 3C7 ascites in ^{125}I -IL-2 binding was 20%. a–h, CTLL mutant clones as indicated; i, EL-4 clone of I-sE; j, parental CTLL-2.

and murine receptors suggest the existence of some functional constraints. Phosphorylation of the serine and threonine residues^{14,15} in the cytoplasmic region also suggests some physiological function for this region. We speculate that phosphorylation of the cytoplasmic region facilitates dissociation of IL-2R from the ternary complex containing the converter and that this is an important step in the feedback regulation of cell growth. Frequent loss of all the mutant receptors expressed on CTLL-2 and EL-4 may support this possibility.

We thank Drs M. Nabholz, E. Shevach and T. Uchiyama for anti-IL-2R antibodies, Dr T. Taniguchi for EL-4 cells and Dr M. Waterfield for the EGFR cDNA clone. Murine and human IL-2 were provided by Ajinomoto Co. and Takeda Chemical Industries, respectively.

Received 30 December 1986; accepted 5 March 1987.

1. Morgan, D. A., Ruscetti, F. W. & Gallo, R. C. *Science* **193**, 1007–1008 (1976).
2. Smith, K. A. *Immunol. Rev.* **51**, 337–353 (1980).
3. Robb, R. J., Greene, W. C. & Rusk, C. M. *J. exp. Med.* **160**, 1126–1146 (1984).
4. Nikaido, T. *et al. Nature* **311**, 631–635 (1984).
5. Leonard, W. J. *et al. Nature* **311**, 626–631 (1984).
6. Cosman, D. *et al. Nature* **312**, 768–771 (1984).
7. Sabe, H. *et al. Molec. Biol. Med.* **2**, 379–396 (1984).
8. Greene, W. C. *et al. J. exp. Med.* **162**, 363–368 (1985).
9. Kondo, S. *et al. Nature* **320**, 75–77 (1986).
10. Hatakeyama, M. *et al. Nature* **318**, 467–469 (1985).
11. Robb, R. J. *Proc. natn. Acad. Sci. U.S.A.* **83**, 3992–3996 (1986).
12. Shimizu, A., Kondo, S., Sabe, H., Ishida, N. & Honjo, T. *Immunol. Rev.* **92**, 103–120 (1986).
13. Kondo, S., Shimizu, A., Saito, Y., Kinoshita, M. & Honjo, T. *Proc. natn. Acad. Sci. U.S.A.* **83**, 9026–9029 (1986).
14. Shackelford, D. A. & Trowbridge, I. S. *J. biol. Chem.* **261**, 8334–8341 (1986).
15. Gallis, B. *et al. J. biol. Chem.* **261**, 5075–5080 (1986).
16. Credig, R., Lowenthal, J. W., Nabholz, M. & MacDonald, H. R. *Nature* **314**, 98–100 (1985).
17. Uchiyama, T., Broder, S. & Waldmann, T. A. *J. Immunol.* **126**, 1393–1397 (1981).
18. Malek, T. R., Robb, R. J. & Shevach, E. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5694–5698 (1983).
19. Fujii, M. *et al. J. Immunol.* **137**, 1552–1556 (1986).
20. Ullrich, A. *et al. Nature* **309**, 418–425 (1984).
21. Zoller, M. J. & Smith, M. *Meth. Enzym.* **100**, 468–500 (1983).
22. Capko, C. L., Roberts, B. E. & Mulligan, R. C. *Cell* **37**, 1053–1062 (1984).

Cross-talk between cellular signalling pathways suggested by phorbol-ester-induced adenylate cyclase phosphorylation

Takaaki Yoshimasa*, David R. Sibley, Michel Bouvier, Robert J. Lefkowitz & Marc G. Caron†

Departments of Medicine (Cardiology), Biochemistry and Physiology, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710, USA

Receptor-mediated activation of both adenylate cyclase and phosphatidylinositol hydrolysis systems occurs through guanine nucleotide regulatory proteins and ultimately leads to specific activation of either cyclic AMP-dependent protein kinase A or Ca^{2+} /phospholipid-dependent protein kinase $\text{C}^{1,2}$. Given the remarkable diversity of agents that influence cellular metabolism through these pathways and the similarities of their components, interactions between the two signalling systems could occur. In fact, stimulation of cells with 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), a phorbol ester that activates protein kinase $\text{C}^{3,4}$, influences hormone-sensitive adenylate cyclase. In some cells TPA induces desensitization of receptor-mediated stimulation of adenylate cyclase^{5,6}, whereas in others, such as frog erythrocytes, phorbol ester treatment results in increased agonist-stimulated as well as basal, guanine nucleotide- and fluoride ion-stimulated adenylate cyclase activities^{7,8}. We show here that TPA produces phosphorylation of the catalytic unit of adenylate cyclase in frog erythrocytes. Moreover, purified protein kinase C can directly phosphorylate *in vitro* the catalytic unit of adenylate cyclase purified from bovine brain. These results suggest that phosphorylation of the catalytic unit of adenylate cyclase by protein kinase C may be involved in the phorbol ester-induced enhancement of adenylate cyclase activity. In addition to providing the first direct demonstration of a covalent modification of the catalytic unit of adenylate cyclase, these results provide a potential biochemical mechanism for a regulatory link between the two major transmembrane signalling systems.

For our present investigation we used the frog erythrocyte system, because in these cells phorbol esters promote a dramatic (100–300%) enhancement of β -adrenergic agonist as well as forskolin- and Mn^{2+} -stimulated adenylate cyclase activities (ref. 7, and data not shown). Forskolin and Mn^{2+} directly stimulate the catalytic unit of adenylate cyclase^{9,10}. This raises the possibility that phorbol-ester-mediated enhancement of adenylate cyclase activity is due to a direct modification of the enzyme. Frog erythrocytes were first preincubated with $^{32}\text{P}_i$, in order to label

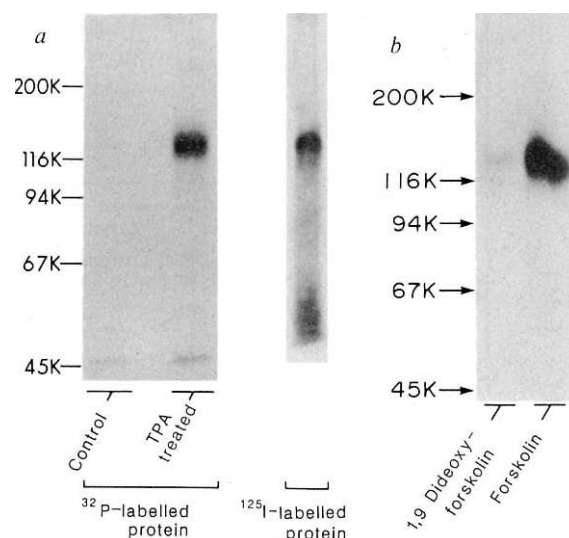
* Present address: Department of Medicine, Tenri Hospital, Nara 632, Japan.

† To whom reprint requests should be addressed.

Fig. 1 Characterization of the adenylate cyclase catalytic unit from control and TPA-treated frog erythrocytes. Size markers, M_r . *a*, SDS-PAGE of ^{32}P -labelled adenylate cyclase from control and TPA-treated frog erythrocytes and of purified radioiodinated adenylate cyclase. *b*, Selective elution of ^{32}P -labelled adenylate cyclase from a forskolin-Sepharose affinity column.

Methods. In *a*, frog erythrocytes were first preincubated with 0.5 mCi ml^{-1} $^{32}\text{P}_i$ to label the intracellular ATP pool as previously described¹⁸, then incubated with $10\text{ }\mu\text{M}$ TPA for 1 h; washing and membrane preparation was as described^{7,18}. In other experiments⁷ we showed that the maximal effect of phorbol esters on the frog erythrocyte adenylate cyclase can be achieved in much shorter times than 1 h (5–10 min). A concentration of $10\text{ }\mu\text{M}$ TPA was used to assure maximal effects. In these cells the 50% effective dose of phorbol ester is about $10\text{--}100\text{ nM}$, which is admittedly lower than the sensitivity of other cell systems. Before solubilization, the washed crude membranes were incubated with $10\text{ }\mu\text{M}$ isoprenaline and $10\text{ }\mu\text{M}$ Gpp(NH)p in preactivation buffer (25 mM Tris-HCl, pH 7.4, 30 mM MgCl_2 , 5 mM EDTA, 1 mM dithiothreitol (DTT), 1 mM NaF, 30 mM Na phosphate, 15 mg ml^{-1} benzamide, $5\text{ }\mu\text{g ml}^{-1}$ soybean trypsin inhibitor, $5\text{ }\mu\text{g ml}^{-1}$ leupeptin and 0.1 mM phenylmethylsulphonylfluoride (PMSF) at 30°C for 30 min to activate adenylate cyclase persistently. The incubation was terminated by diluting with 2–3 vol ice-cold wash buffer (same as preactivation buffer except that the concentration of MgCl_2 was 10 mM) and centrifugation at $30,000\text{ g}$ for 10 min. The preactivated membranes were washed once in the same buffer.

The preactivation procedure improved recoveries of the subsequent solubilization and affinity chromatography steps. In addition, preactivation and solubilization render the enzyme maximally active so that the activities of control and phorbol-ester-treated enzyme become equivalent. Thus, the specific activities of both preparations assayed under these conditions can be assumed to be the same. The membranes were suspended in 3% Lubrol PX in wash buffer, stirred at 4°C for 30 min, and then centrifuged at $45,000\text{ g}$ for 60 min. The soluble preparation was purified by sequential affinity chromatography on forskolin-Sepharose and wheat-germ agglutinin-agarose as described¹¹. Briefly, the soluble extract was applied to 5 ml of a forskolin-Sepharose column pre-equilibrated with 3 column vols of chromatography buffer (wash buffer containing 1 mM Tween 60 and 100 mM NaCl). The column was washed with 1 column vol. of high-salt wash buffer (same as chromatography buffer except that 100 mM NaCl is replaced by 1 M NaCl) and then one column vol. of chromatography buffer. The adenylate cyclase was eluted specifically with 2 column vols of the same buffer including $100\text{ }\mu\text{M}$ forskolin. The eluate was directly applied to a 1-ml column of wheat-germ agglutinin-agarose, washed and then eluted with 2 column vols of chromatography buffer containing 0.1 M *N*-acetyl-glucosamine. A $20\text{-}\mu\text{l}$ aliquot of each eluate was assayed for adenylate cyclase activity in the presence of 5 mM MgCl_2 and $100\text{ }\mu\text{M}$ forskolin. For the experiment shown, control activity was $6.3\text{ pmol cAMP min}^{-1}$ whereas the TPA-treated enzyme activity was $7.0\text{ pmol cAMP min}^{-1}$. The rest of the eluate was lyophilized and redissolved in 0.25 ml of electrophoresis sample buffer. SDS-PAGE was performed by the method of Laemmli²⁵ using 6% homogeneous slab gels. After autoradiography, the enzyme bands were sliced out of the gel and the ^{32}P -label was quantified by liquid scintillation spectroscopy. For the autoradiogram shown at right, adenylate cyclase was purified from control cells by the same procedure as that used in ^{32}P incorporation experiments. After purification, samples were radioiodinated using ^{125}I -labelled Bolton-Hunter reagent as described²⁶. Radioiodinated adenylate cyclase was also electrophoresed on a 6% polyacrylamide gel. In *b*, frog erythrocytes were ^{32}P -labelled and then incubated with $10\text{ }\mu\text{M}$ TPA for 1 h at 23°C . The adenylate cyclase was solubilized and applied to the forskolin-Sepharose column. The column was first eluted with buffer containing $100\text{ }\mu\text{M}$ 1,9-dideoxyforskolin (lane 1) and subsequently with buffer including $100\text{ }\mu\text{M}$ forskolin (lane 2). The eluates were further purified on 1 ml of wheat-germ agglutinin-agarose. The eluates from wheat-germ agglutinin-agarose were assayed ($20\text{ }\mu\text{l}$) for enzyme activity as described above before electrophoresis on a 6% polyacrylamide gel. Enzyme activities for the experiment shown were $0.19\text{ pmol cAMP per min}$ for the 1,9-dideoxyforskolin eluate and 2.4 pmol min^{-1} for the forskolin eluate. This experiment was performed twice with similar results each time.



the intracellular ATP pool, then incubated with phorbol esters. Membranes were prepared and preactivated with isoprenaline and Gpp(NH)p before solubilization of adenylate cyclase with Lubrol PX. Adenylate cyclase was then purified by sequential affinity chromatography on forskolin-Sepharose and wheat-germ agglutinin-agarose gels as previously described¹¹ and subsequently analysed by SDS-polyacrylamide gel electrophoresis (PAGE). Virtually no phosphopeptides were observed in control preparations after autoradiography of SDS-PAGE-analysed material (Fig. 1*a*, left panel). In preparations from TPA-treated cells, however, a phosphoprotein with an apparent relative molecular mass of 130,000 (M_r 130K) was observed (Fig. 1*a*). Note that essentially equal amounts of enzyme were loaded onto the two gel lanes.

The M_r of the phosphopeptide is consistent with reported values for the enzyme purified from various tissues, which have ranged from 120K to 160K (refs 11, 12–16). However, we attempted to identify more rigorously the 130K phosphoprotein as the catalytic unit of the frog erythrocyte adenylate cyclase. First, we purified the adenylate cyclase by the same procedure used in the ^{32}P incorporation experiments, and followed this with radioiodination of the purified preparation and analysis by SDS-PAGE. As shown in Fig. 1*a*, (right), SDS-PAGE of the radioiodinated protein revealed a single predominant band that co-migrated with the phosphopeptide of M_r 130K. Further evidence that the 130K phosphopeptide represents the adenylate cyclase catalytic subunit is shown in Fig. 1*b*. Solubilized enzyme

from ^{32}P -labelled TPA-treated cells was applied to a forskolin-Sepharose affinity column. The column was first eluted with 1,9-dideoxyforskolin, which has been shown neither to activate adenylate cyclase nor to compete for ^3H -forskolin binding in rat brain¹⁷. Almost no enzyme activity was eluted from the column and, as can be seen in Fig. 1*a*, lane 1, the phosphoprotein of interest is absent. When the column was then eluted specifically with forskolin, the enzyme activity and the phosphoprotein appeared in the eluate (Fig. 1*b*, lane 2). The data in Fig. 1 thus indicate that the 130K phosphoprotein is in fact the catalytic unit of the frog erythrocyte adenylate cyclase and that this peptide is phosphorylated after treatment of cells with phorbol esters.

To estimate the stoichiometry of adenylate cyclase phosphorylation, the amount of enzyme loaded on the gel lanes was determined from the total activity in each preparation. The specific activity of each enzyme preparation loaded on the gel was assumed to be $1.5\text{ }\mu\text{mol cAMP min}^{-1}\text{ mg}^{-1}$, because the apparent purity of the preparations evaluated by radioiodination was $>90\%$ (Fig. 1*a* and legend). This value is representative of the specific activity of the enzyme purified from bovine brain¹¹. Protein concentrations of the purified erythrocyte adenylate cyclase preparations were too low to be quantified. After quantification of ^{32}P incorporation, the specific activity of the ^{32}P -labelled enzyme was divided by the specific activity of the intracellular $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (determined as in ref. 18) to arrive at a phosphate/enzyme stoichiometry. The stoichiometry of phos-

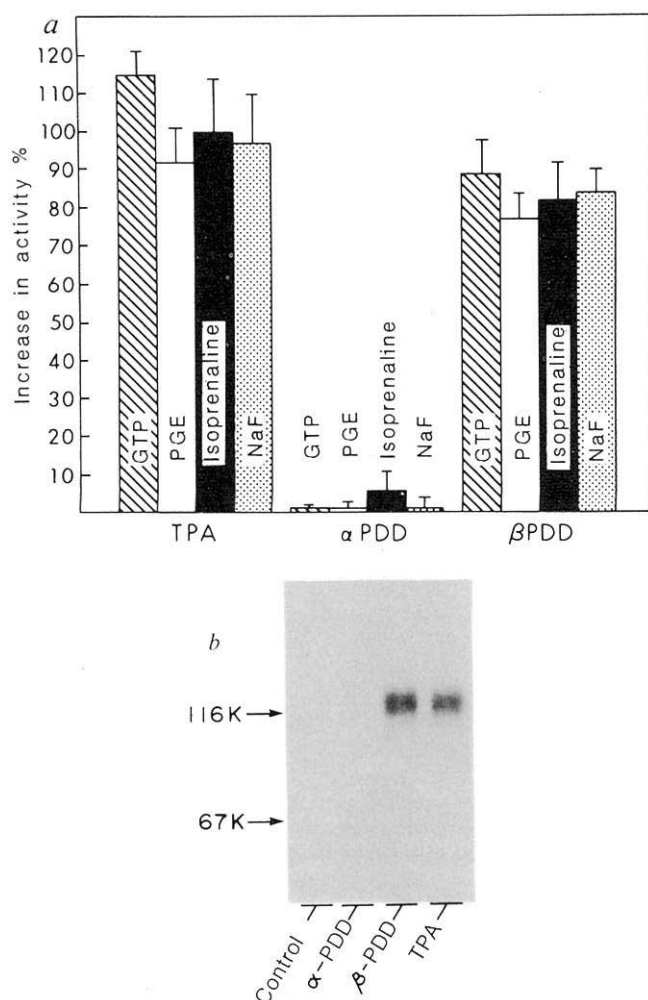


Fig. 2 Pharmacological specificity of phorbol-ester-induced sensitization (a) and phosphorylation (b) of frog erythrocyte adenylate cyclase. Frog erythrocytes were preincubated with $^{32}\text{P}_i$ and then incubated with $10\ \mu\text{M}$ concentrations of TPA, α -PDD and β -PDD for 1 h as in Fig. 1. a, Before preactivation and solubilization, adenylate cyclase activities were determined as described⁵. The data shown are expressed as the percentage increase in adenylate cyclase activity compared to control values with GTP (hatched bars), PGE₁ (open bars), isoprenaline (filled bars) and NaF (stippled bars). Typical control values were 3.1 (GTP), 16 (PGE₁), 100 (isoprenaline), 330 (NaF) pmol cAMP min⁻¹ mg⁻¹. b, Before purification, the samples were electrophoresed on a 6% polyacrylamide gel as in Fig. 1. Enzyme activities for the experiment shown were 8.3 (control), 7.2 (α -PDD), 9.2 (β -PDD), 8.9 (TPA) pmol cAMP min⁻¹ when assayed as described in Fig. 1. This experiment was performed twice with similar results each time. Size markers, M_r .

phorylation of the enzyme by this procedure gave a value of 3.3 ± 0.72 (mean $\pm$ s.e.m., $n = 3$) mol of phosphate per mol of catalytic subunit for the TPA-sensitized enzyme. Under these conditions, no phosphate was detected in the enzyme derived from control cells.

Figure 2 shows the pharmacological specificity of enzyme sensitization (Fig. 2a) and the accompanying phosphorylation (Fig. 2b) of the adenylate cyclase catalytic subunit induced by phorbol esters. In addition to TPA, 4 β -phorbol 12,13-didecanoate (β -PDD), which is a potent tumour promotor and activator of protein kinase C⁴, was effective in promoting enhanced GTP-, prostaglandin E₁ (PGE₁)-, isoprenaline- and NaF-stimulated adenylate cyclase activity and catalytic unit phosphorylation. In contrast, the stereoisomer of β -PDD, 4 α -phorbol 12,13-didecanoate (α -PDD), which is devoid of

tumour-promoting ability and does not activate protein kinase C⁴, was not active in sensitizing adenylate cyclase activity or in promoting phosphorylation of the enzyme. Thus, the sensitization and phosphorylation of the enzyme induced by phorbol esters is pharmacologically specific and appears to be due to stimulation of protein kinase C activity.

To obtain more direct evidence for the involvement of protein kinase C in phorbol-ester-mediated sensitization and phosphorylation of adenylate cyclase we examined the ability of purified protein kinase C to phosphorylate the pure catalytic unit of adenylate cyclase *in vitro*. Figure 3 presents a time course in which the purified bovine brain catalytic unit (M_r 160K) is incubated in the presence of purified protein kinase C. Under these conditions the enzyme becomes phosphorylated to a stoichiometry of about 1 mol of phosphate per mol of enzyme and phosphorylation is nearly maximum at the earliest point examined, 5 min. These results indicate that adenylate cyclase is a substrate for protein kinase C *in vitro* and are therefore consistent with the whole-cell effects of phorbol esters being mediated by a direct effect of protein kinase C on the enzyme. Although the stoichiometry of the *in vitro* phosphorylated enzyme (bovine brain) differs from that observed in the intact frog erythrocyte this difference may reflect in part slight variations in the specific activity of the enzyme from these two tissues.

In this study we clearly demonstrate in an intact cell system that the catalytic unit of adenylate cyclase can undergo stoichiometric phosphorylation accompanied by sensitization of enzyme activity. This result represents the first direct demonstration of the involvement of covalent modification (phosphorylation) of the catalytic unit of adenylate cyclase in the regulation of hormone responsiveness. The phosphorylation is highly specific in that only those phorbol esters which activate protein kinase C are capable of promoting adenylate cyclase sensitization and catalytic unit phosphorylation. The putative function of protein kinase C in mediating this effect is further strengthened by the demonstration that purified protein kinase C can phosphorylate the pure catalytic unit of adenylate cyclase *in vitro*. In future studies it will be of interest to document whether such covalent modification of the enzyme *in vitro* enhances its activity and to examine which parameters of enzyme responsiveness might be affected. This may well require reinsertion of the enzyme in lipid vesicles and reconstitution of its interactions with other components of the system such as the β -adrenergic receptor and the guanine nucleotide regulatory protein.

It has recently been speculated that the observed enhancement of adenylate cyclase activity in phorbol-ester-treated cells may be due to an impairment in function of G_i, the inhibitory guanine nucleotide regulatory protein¹⁹⁻²¹. The α -subunit of G_i is a substrate for protein kinase C *in vitro*²⁰, but protein kinase C-induced phosphorylation of this G_i α -subunit was not observed to be stoichiometric and the functional activity of the phosphorylated protein was not directly tested²⁰. Moreover, there has been no report of G_i phosphorylation in any intact cell system. Although our data do not rule out an additional effect of protein kinase C on the G_i inhibitory pathway, phosphorylation of the adenylate cyclase catalytic unit appears to be sufficient to explain the observed phorbol-ester-induced sensitization effect.

The protein kinase C-mediated phosphorylation of the adenylate cyclase catalytic unit may provide a mechanism by which the adenylate cyclase and phosphatidylinositol transmembrane signalling systems interact. Several recent reports showing that activation of the phosphatidylinositol turnover pathway leads to sensitization of the adenylate cyclase system seem to provide support for the possible physiological relevance of the proposed mechanism^{22,23}. Sugden *et al.*²² have shown that stimulation of α_1 -adrenergic receptors in rat pinealocytes potentiates isoprenaline-induced cyclic AMP accumulation in these cells. On the

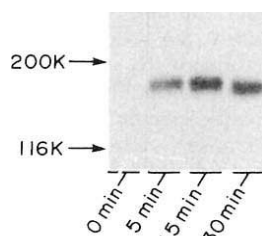


Fig. 3 Time course of phosphorylation of adenylate cyclase by protein kinase C. Size markers, M_r .

Methods. Bovine brain (caudate) adenylate cyclase was purified by modification¹¹ of the procedures described in Fig. 1 legend. The final material was >95% pure as judged by its specific activity ($1.5 \mu\text{mol min}^{-1} \text{mg}^{-1}$) and SDS-PAGE¹¹. The 160K peptide of adenylate cyclase obtained by this procedure was incubated with protein kinase C in the presence of 10 mM Tris-HCl, pH 7.2, 100 mM NaCl, 6 mM MgCl_2 , 0.1 mM CaCl_2 , 0.1 mg ml^{-1} phosphatidylserine, 25 mM NaH_2PO_4 , 0.1 mM TPA and 0.025 mM [γ - ^{32}P]ATP ($\sim 2,500 \text{ c.p.m. pmol}^{-1}$) at 25 °C for the indicated periods. The protein kinase C was prepared, as described by Uchida *et al.*, from porcine brain²⁷. Because the adenylate cyclase was stored in 5 mM Tween 60, phosphorylation reactions were conducted in mixed micelles (phosphatidylserine/Tween 60) and the TPA concentration (0.1 mM) was chosen to give >1 molecule of TPA per micelle. The kinase activity was >95% dependent on CaCl_2 , phosphatidylserine and TPA and the preparation was free of cAMP, cGMP- or Ca^{2+} /calmodulin-dependent protein kinase activity. Reactions were stopped by dilution with ice-cold 100 mM NaCl, 10 mM Tris-HCl, pH 7.4 and phosphorylated samples were further purified on a 0.5 ml wheat-germ agglutinin-agarose column to remove contaminating phosphoproteins. The samples were then electrophoresed on a 6% SDS-polyacrylamide gel. After drying and autoradiography the gels were cut and counted to determine the stoichiometry.

other hand, Nabika *et al.*²³ have found that in vascular smooth muscle cells, angiotensin II also enhances cyclic AMP accumulation stimulated by isoprenaline and vasoactive intestinal peptide. To probe the possible physiological significance of the phosphorylation mechanism proposed here, such cell systems might be useful models. They could be used to examine directly whether effects on responsiveness of adenylate cyclase mediated by receptors coupled to phosphatidylinositol breakdown can be correlated with phosphorylation of the enzyme. Together with previous findings on the regulation of receptor function by phosphorylation²⁴, the results presented in this paper delineate further the complexity and variety of mechanisms that have evolved to regulate cellular sensitivity to hormone and drug actions.

We thank Donna Addison for help with the manuscript and Dr K. B. Seamon, NIH, for the 1,9-dideoxyforskolin.

Received 16 January; accepted 2 March 1987.

1. Nishizuka, Y. *Nature* **308**, 693-698 (1984).
2. M. Berridge & Irvine, R. F. *Nature* **312**, 315-321 (1984).
3. Castagna, M. *et al.* *J. biol. Chem.* **257**, 7849-7857 (1982).
4. Kikkawa, U., Takai, Y., Tanaka, Y., Miyake, R. & Nishizuka, Y. *J. biol. Chem.* **258**, 11442-11445 (1983).
5. Sibley, D. R., Nambi, P., Peters, J. R. & Lefkowitz, R. J. *biochem., Biophys. Res. Commun.* **121**, 973-979 (1984).
6. Kelleher, D. J., Pessin, J. E., Ruoho, A. & Johnson, G. L. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4316-4320 (1984).
7. Sibley, D. R., Jeffs, R. A., Daniel, K., Nambi, P., & Lefkowitz, R. J. *Archs Biochem. Biophys.* **244**, 373-381 (1986).
8. Bell, J. D., Buxton, I. L. & Brunton, L. L. *J. biol. Chem.* **260**, 2625-2628 (1985).
9. Seamon, K. B., Padgett, W. & Daly, J. W. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3363-3367 (1981).
10. Bender, J. L. & Neer, E. J. *J. biol. Chem.* **258**, 2432-2439 (1983).
11. Yoshimasa, T. *et al.* *J. biol. Chem.* (submitted).
12. Pfeuffer, E., Drehe, R.-M., Metzger, H. & Pfeuffer, T. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3086-3090 (1985).
13. Cousson, F., Haiech, J., D'Alayer, J. & Monneron, A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6736-6740 (1985).
14. Pfeuffer, E., Mollner, S. & Pfeuffer, T. *EMBO J.* **4**, 3675-3679.
15. Yeager, R. E., Heideman, W., Rosenberg, G. B. & Storm, D. R. *Biochemistry* **24**, 3776-3783 (1985).
16. Smigel, M. D. *J. biol. Chem.* **261**, 1976-1982 (1986).

17. Seamon, K. B., Vaillancourt, R., Edwards, M. & Daly, J. W. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5081-5085 (1984).
18. Sibley, D. R., Strasser, R. H., Caron, M. G. & Lefkowitz, R. J. *J. biol. Chem.* **260**, 3883-3886 (1985).
19. Jakobs, K. H., Bauer, S. & Watanabe, Y. *Eur. J. Biochem.* **151**, 425-430 (1985).
20. Katada, T., Gilman, A. G., Watanabe, Y., Bauder, S. & Jakobs, K. H. *Eur. J. Biochem.* **151**, 431-437 (1985).
21. Olanas, M. C. & Onali, P. *J. Neurochem.* **47**, 890-897 (1986).
22. Sugden, D., Vanecek, J., Klein, D. C., Thomas, T. P. & Anderson, W. B. *Nature* **314**, 359-361 (1985).
23. Nabika, T., Nara, Y., Yamori, Y., Lovenberg, W. & Endo, J. *Biochem. biophys. Res. Commun.* **131**, 30-36 (1985).
24. Sibley, D. R. & Lefkowitz, R. J. *Nature* **317**, 124-129 (1985).
25. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
26. Bolton, A. E. & Hunter, W. M. *Biochem. J.* **133**, 529-530 (1973).
27. Uchida, T. & Filburn, C. R. *J. biol. Chem.* **258**, 12311-12314 (1984).

High-velocity microprojectiles for delivering nucleic acids into living cells

T. M. Klein*, E. D. Wolf†, R. Wu‡ & J. C. Sanford*

* Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456, USA

Departments of † Electrical Engineering and ‡ Biochemistry, Cornell University, Ithaca, New York 14853, USA

We report here a novel phenomenon, namely that nucleic acids can be delivered into plant cells using high-velocity microprojectiles. This research was conducted in the hope of circumventing some of the inherent limitations of existing methods for delivering DNA into plant cells¹⁻⁶. After being accelerated, small tungsten particles (microprojectiles) pierce cell walls and membranes and enter intact plant cells without killing them. Microprojectiles were used to carry RNA or DNA into epidermal tissue of onion and these molecules were subsequently expressed genetically. This approach can therefore be used to study the transient expression of foreign genes in an intact tissue. It remains to be shown that smaller cell types, as are found in regenerable plant tissues, can be stably transformed by this method. If this proves possible, it would appear to provide a broadly applicable transformation mechanism capable of circumventing the host-range restrictions of *Agrobacterium tumefaciens*¹, and the regeneration problems of protoplast transformation²⁻⁵.

Several devices for accelerating small particles to high velocities have been designed and tested⁷. The device (particle gun) illustrated in Fig. 1a was used to accelerate tungsten microprojectiles (spherical particles 4 μm in diameter) into intact epidermal cells of *Allium cepa* (onion) (Fig. 1b and c). Many cells can be bombarded simultaneously and about 90% of the cells in a 1-cm² of *A. cepa* epidermal tissue ($\sim 2,000$ cells) typically contain microprojectiles following bombardment. Cells from *A. cepa* can survive penetration by many microprojectiles (Fig. 1d). However, the viability of cells (as determined by the maintenance of cytoplasmic streaming for at least 24 h after bombardment) is adversely affected by penetration by a large number of microprojectiles (Fig. 2).

To demonstrate that nucleic acid can be delivered into cells by this method, RNA isolated from tobacco mosaic virus (TMV) strain U₁ was adsorbed to the surface of 4- μm tungsten particles before their acceleration into *A. cepa* cells. Expression of the viral RNA was monitored by examining the bombarded cells microscopically for the presence of viral inclusion bodies (crystallized virus particles⁸) 3 days after treatment. Crystalline material, in the form of hexagonal plates, round plates and needles, was observed in the cytoplasm and vacuole of 30-40% of the cells that contained microprojectiles (Table 1). These distinctive crystalline inclusions were never observed in un-bombarded tissue or in tissue bombarded with microprojectiles containing no nucleic acid. The observed percentage of cells expressing TMV following bombardment was comparable to

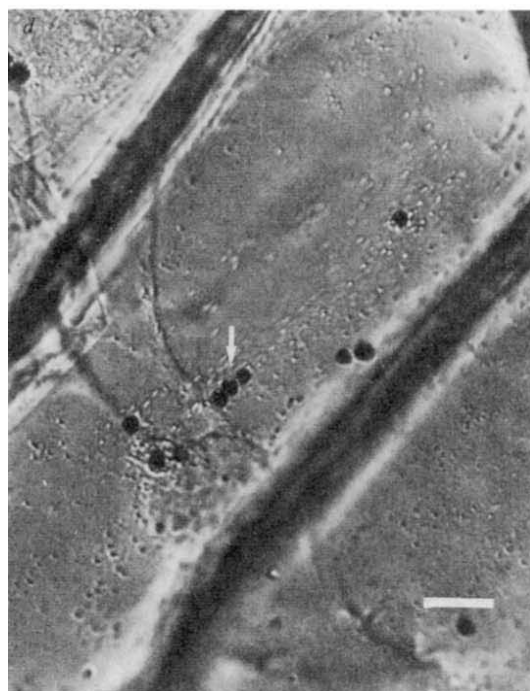
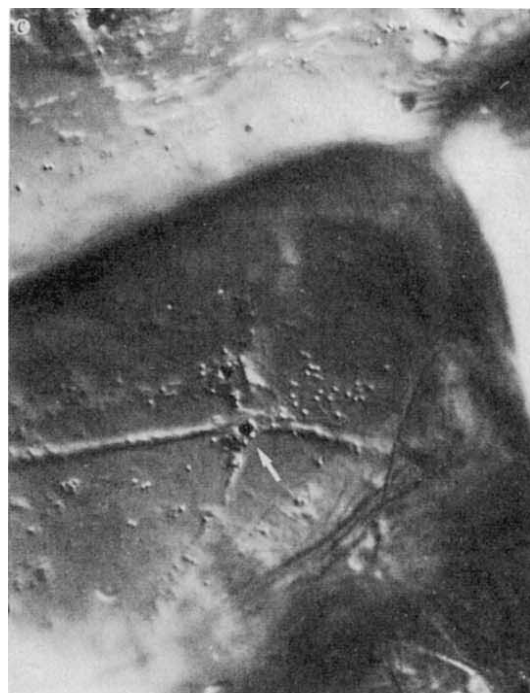
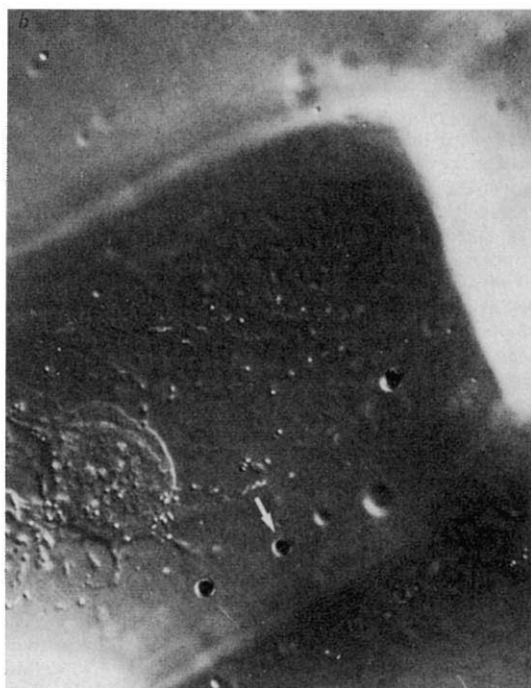
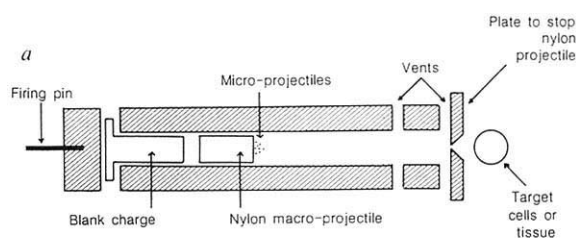


Fig. 1 *a*, Schematic diagram of the particle gun. About 0.05 mg of tungsten particles (average diameter 4 μm , General Electric Corp., Cleveland, Ohio) is placed on the front surface of a cylindrical nylon projectile (diameter, 5 mm; length, 8 mm) as a suspension in 1–2 μl of water. A gun powder charge (GY22AC, gray extra light, No. 1, Speed Fasteners Inc., St Louis, Missouri), detonated with a firing pin, is used to accelerate the nylon projectile down the barrel of the device. The tungsten particles continue toward the target cells through a small 1-mm aperture in a steel plate designed to stop the nylon projectile. The tungsten microprojectiles leave the particle gun with an initial velocity of about 430 m s^{-1} . This value was determined by allowing the nylon macroprojectile to leave the barrel of the device and estimating its velocity in-flight with a chronograph (Ohler Research, Austin, Texas). The target cells are placed ~10–15 cm from the end of the device. *b–d*, Nomarski micrographs of *A. cepa* epidermal cells following bombardment with tungsten microprojectiles 4 μm in diameter. The epidermal layer was stripped from the underlying bulb tissue before bombardment. *b*, Cell with 3 microprojectiles on its surface (one arrowed); *c*, microprojectile (arrowed) within the same cell as revealed by focusing 40 μm below the surface of the cell; *d*, Eight microprojectiles (one arrowed) in the interior of a living cell. Scale bar, 20 μm .

that reported for the delivery of TMV RNA into protoplasts by liposome uptake^{9,10} or electroporation¹¹.

Using the particle gun, DNA can be delivered into intact plant cells, and this can result in transient expression of a foreign gene. Tungsten microprojectiles were coated with plasmid (p35S-CAT)¹² containing a gene that encodes chloramphenicol acetyltransferase (CAT). These microprojectiles were used to bombard 1-cm² sections of *A. cepa* epidermal tissue. Extracts from epidermal tissue bombarded with microprojectiles coated with p35S-CAT showed very high levels of CAT activity with most of the chloramphenicol being converted to its acetylated derivatives during the assay (Fig. 3). Strong CAT activity was detected in extracts from as little as a single 1-cm² piece of bombarded epidermal tissue (~2,000 cells) whereas control tissue showed negligible CAT activity.

These findings indicate that particle bombardment can be used to deliver RNA or DNA into large numbers of intact plant cells simultaneously and that the foreign nucleic acids introduced by this process can subsequently be expressed. The introduction of DNA into *A. cepa* epidermal tissue by the particle bombardment process should be a useful tool for the study of transient expression of foreign nucleic acids in plant cells. The process does not require cell culture or the pretreatment of the recipient tissue in any way and only small quantities of DNA are needed (about 0.1 μg per treatment). Because the preparation of particles and the bombardment of tissue can be rapid, many nucleic acid samples can be tested in a short period of time.

Particle bombardment may eventually prove useful for the transformation of other plant species and may be of particular

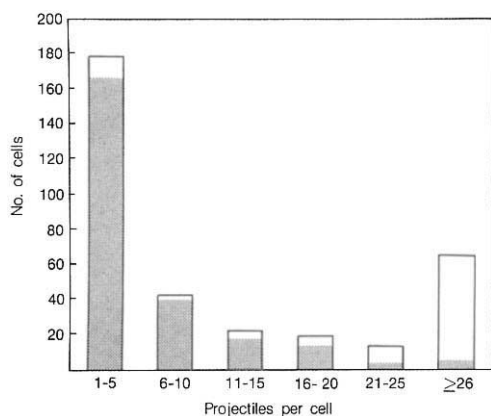


Fig. 2 Relationship between cell survival and the number of microprojectiles within a cell. Bars (solid lines), total cells; shaded part of bar, living cells. Three 1-cm² sections of *A. cepa* epidermal tissue were bombarded as described in Fig. 1. About 100 cells from each section were analysed microscopically for viability (based on the maintenance of cytoplasmic streaming) and the number of microprojectiles in these cells were counted. Data from the three sections of tissue were pooled.

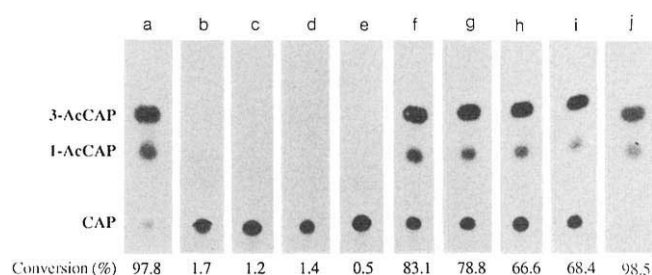


Fig. 3 CAT activity from *A. cepa* epidermal tissue bombarded with microprojectiles coated with plasmid harbouring the CAT gene. Lane a, positive control: a colony of *E. coli* carrying pBR325 was sonicated in 1 ml of buffer and centrifuged at 10,000g for 5 min. The supernatant (10 µl) was used for the CAT assay. Lane b, untreated epidermal tissue (10 pooled sections); lane c, epidermal tissue (10 pooled sections) bombarded with naked microprojectiles; lane d, epidermal tissue (10 pooled sections) bombarded with microprojectiles coated with pUC13. Lane e, microprojectiles (30 µl of a suspension prepared as described in the text) were rubbed over the surface of each of 10 sections of epidermal tissue which were pooled for the assay. Lane f-i, epidermal tissue (10 sections pooled for each assay) bombarded with microprojectiles coated with p35S-CAT; lane j: epidermal tissue (one section for the assay) bombarded with p35S-CAT. CAP, chloramphenicol; 3-AcCAP and 1-AcCAP, monoacetylated forms of chloramphenicol. The plasmid (p35S-CAT), which has been previously described¹², includes the following components: a 941-basepair (bp) region including the 35S promoter from cauliflower mosaic virus²³; a 900-bp region encoding the CAT activity²⁴; a 700-bp region carrying the 3' noncoding sequences and the transcription termination region of the ribulose biphosphate carboxylase gene and a derivative of the pUC13 plasmid in which the *HincII* site was changed to a *ClaI* site. The resulting construct is 5.3 kilobases (kb) long. The plasmid p35S-CAT was grown in *Escherichia coli*, isolated by phenol extraction, and purified by CsCl/ethidium bromide density-gradient centrifugation²⁵. The microprojectiles were coated by adding 10 µl of the plasmid DNA (1.0 µg of DNA per µl of TE buffer, pH 7.7) to 100 µl of a suspension of tungsten microprojectiles (0.1 g of tungsten per ml of distilled water). The DNA was precipitated by the addition of 100 µl of a CaCl₂ solution (2.5 M) and 40 µl of a spermidine solution (free base, 0.1 M). The resulting suspension was centrifuged for 30 min at 13,000g. *A. cepa* epidermal tissue (1-cm² sections) was then bombarded with the DNA-coated microprojectiles (2 µl of the suspension placed in the middle of the front surface of the nylon macroprojectile). Assays of CAT activity were performed on extracts prepared from tissue that had been incubating for 3 days following bombardment with the microprojectiles. Tissue extracts were prepared by placing the epidermal sections in an Eppendorf tube (1.5 ml) with 100 µl Tris-HCl (0.25 M, pH 7.8) and grinding the tissue with a pestle mixer (Kontes). CAT activity in the extracts was determined in 30-min assays as previously reported²⁶, except that the amount of ¹⁴C-chloramphenicol was decreased to 0.1 µCi per assay¹⁸. All the radioactivity in the Eppendorf tube (~170,000 c.p.m. in each assay) was spotted on the thin-layer chromatography (TLC) plate (Chromagram, Kodak). After resolution of chloramphenicol and its acetylated derivatives by chromatography, an autoradiogram (12-h exposure) was made. Quantitative results were obtained by scintillation counting of separated spots of chloramphenicol and its acetylated derivatives, and the conversion calculated.

Table 1 The delivery of TMV RNA to *A. cepa* cells by accelerated microprojectiles

Trial	Cells containing microprojectiles:		Proportion penetrated cells with inclusion bodies (%)
	without inclusions	with inclusions	
1	44	34	43.6
2	85	35	29.2
3	168	98	36.8
4	257	76	22.8
5	147	52	26.1

A. cepa epidermal cells were scored for presence of TMV inclusion bodies following bombardment with microprojectiles to which TMV RNA has previously been adsorbed. About 50 microscope fields (×500) were observed within a 1-cm² area of tissue for each trial.

Methods. For adsorption of RNA to the tungsten microprojectiles, 2 µl of a TMV RNA²¹ solution (2 µg of RNA per µl of distilled water) were added to 18 µl of a suspension of tungsten microprojectiles (10 mg of tungsten per ml of distilled water). The RNA was precipitated by the addition of 7.5 µl of 0.25 M CaCl₂ solution and the resulting suspension was centrifuged for 30 min at 13,000g. The particles were resuspended and 2 µl of the suspension was placed on the front surface of the nylon projectile. Centrifugation brings the RNA precipitate into close association with the tungsten surfaces and results in uniform adsorption which can be visualized by staining with the fluorescent dye DAPI²². Following bombardment the tissue was incubated for 3 days at 21 °C.

value for those species that cannot be genetically engineered successfully with existing techniques. Although several delivery systems have been used to transfer genes into various dicotyledonous plant species^{1,2,13,14}, these techniques have not yet been useful for the production of whole, transformed plants of graminaceous species such as rice, wheat, or corn. Although there is evidence that *Agrobacterium* can transfer DNA to *Zea mays*¹⁵, *Asparagus*¹⁶, *Chlorophytum* and *Narcissus*¹⁷, the efficient use of this agent for the transfer of genes to monocots may be hampered by the restricted host range of the bacterium¹. Genes have been transferred to protoplasts of the monocots *Triticum monococcum* and *Lolium multiflorum* by incubation with plasmid DNA^{3,4} and to *Zea mays*⁵ and *Oryza sativa*¹⁸ by electroporation, but these techniques are restricted by the difficulties of regenerating whole plants from protoplasts of most monocot species^{19,20}. The particle bombardment process should not suffer from the host-range limitations of infectious agents

such as *Agrobacterium*, and the problems associated with systems that require regeneration of plants from protoplasts might be circumvented by bombarding regenerable tissues such as meristems or embryogenic callus with DNA-bearing microprojectiles. To accomplish this, the particle bombardment process must be refined so that cells much smaller (10–20 µm) than those of *A. cepa* epidermal tissue can be penetrated by microprojectiles.

This work was supported by grants from the Cornell Biotechnology Program, the US Department of Agriculture, and the Rockefeller Foundation. We thank N. Allen for input on the

design of the particle gun and its construction, M. Zaitlin and M. Nishiguchi for TMV RNA, and M. McCann for technical assistance with the CAT assays.

Received 7 January; accepted 2 March 1987.

1. Fraley, R. T., Rogers, S. G. & Horsch, R. B. *CRC crit. Rev. Pl. Sci.* **4**, 1-46 (1986).
2. Steinbiss, H. H. & Broughton, W. *Int. Rev. Cytol. (Suppl.)* **16**, 191-207 (1983).
3. Lörz, H., Baker, B. & Schell, J. *Molec. gen. Genet.* **199**, 178-182 (1985).
4. Potrykus, I., Saul, M. V., Petruska, J., Paszkowski, J. & Shillito, J. R. D. *Molec. gen. Genet.* **199**, 183-188 (1985).
5. Fromm, M., Taylor, L. P. & Walbot, V. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5824-5828 (1985).
6. De la Peña, A., Lörz, H. & Schell, J. *Nature* **325**, 274-276 (1987).
7. Sanford, J. C., Klein, T. M., Wolf, E. D. & Allen, N. *Particle Sci. Technol.* (in the press).
8. Christie, R. G. & Edwardson, J. R. *Light and Electron Microscopy of Plant Virus Inclusions, Florida Agric. Exp. Station Monograph Ser. 9* (University of Florida, Gainesville, 1977).
9. Fukunaga, Y., Nagata, T. & Takebe, I. *Virology* **113**, 752-760 (1981).
10. Fraley, R. T., Dellaporta, S. L. & Papahadjopoulos, D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1859-1863 (1982).
11. Nishiguchi, M., Langridge, W. H. R., Szalay, A. A. & Zaitlin, M. *Pl. Cell Rep.* **5**, 57-60 (1986).
12. Morelli, G., Nagy, F., Fraley, R. T., Rogers, S. G. & Chua, N.-H. *Nature* **315**, 200-204 (1985).
13. Crossway, A. *et al. Molec. gen. Genet.* **202**, 179-185 (1986).
14. Brisson, N. & Hohn, T. *Meth. Enzym.* **118**, 659-668 (1986).
15. Grimsley, N., Hohn, T., Davies, J. W. & Hohn, B. *Nature* **325**, 177-179 (1987).
16. Hernalsteens, J.-P., Thia-Toong, L., Schell, J. & Van Montagu, M. *EMBO J.* **3**, 3039-3041 (1984).
17. Hooykaas-Van Slooter, G. M. S., Hooykaas, P. J. J. & Schilperoord, R. A. *Nature* **311**, 763-764 (1984).
18. Ou-Lee, T. M., Turgeon, R. & Wu, R. *Proc. natn. Acad. Sci. U.S.A.* **83**, 6815-6819 (1986).
19. Vasil, I. K. *Int. Rev. Cytol. (Suppl.)* **16**, 79-88 (1983).
20. Potrykus, I. & Shillito, R. F. *Meth. Enzym.* **118**, 549-578 (1986).
21. Zaitlin, M. in *Nucleic Acids in Plants Vol. 2* (eds Hall, T. C. & Davis, J. W.) 31-64 (CRC Press, Boca Raton, Florida, 1979).
22. Coleman, A. W. & Goff, J. L. *Stain Technol.* **60**, 145-154 (1985).
23. Hohn, T., Richards, K. & Lebeurier, G. *Current Topics Microbiol. Immun.* **96**, 193-236 (1982).
24. Alton, N. K. & Vapnek, D. *Nature* **282**, 864-869 (1979).
25. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning, a Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
26. Gorman, C. M., Moffat, L. F. & Howard, B. H. *Molec. cell. Biol.* **2**, 1044-1051 (1982).

Determination of bacteriophage λ tail length by a protein ruler

Isao Katsura

Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan

How the size and shape of living structures are determined by genetic information is one of the fundamental problems in biology. Here I describe a study in which the size of a biological supramolecular structure was changed in a predictable way by *in vitro* genetics, with the size both before and after manipulation being exactly determined. I have studied the tail of bacteriophage λ , whose length is determined by the length of the 'ruler protein', the product of gene *H*. The length of the tail can be decreased or increased by deleting the middle part of gene *H* or by forming a small duplication there, and the length of the tail is proportional to the size of the protein. These results can be regarded as a special case of protein engineering¹, namely supramolecular protein engineering.

Previous work² has shown that some λ mutants *in vivo* that have small in-frame deletions in gene *H* produce viable phage particles with a tail slightly shorter than wild-type. This proves that gene *H* determines the tail length and gives strong evidence for a scheme in which its product (abbreviated as gpH) is a ruler that measures length during tail assembly. The question arose from that work regarding the structure-function relationship of gpH, which has at least three functions: tail assembly, tail-length determination and DNA injection². It also led to interest in the relationship between the length of gpH and the length of the tail, and whether it is possible to change the tail length over a much wider range of sizes.

In this study, deletion and duplication mutants in gene *H* of λ phage were isolated in the following way. First, a fragment of λ DNA containing gene *H* was cloned in the plasmid pBR322 (ref. 3). Then, either a deletion (of one of a number of sizes)

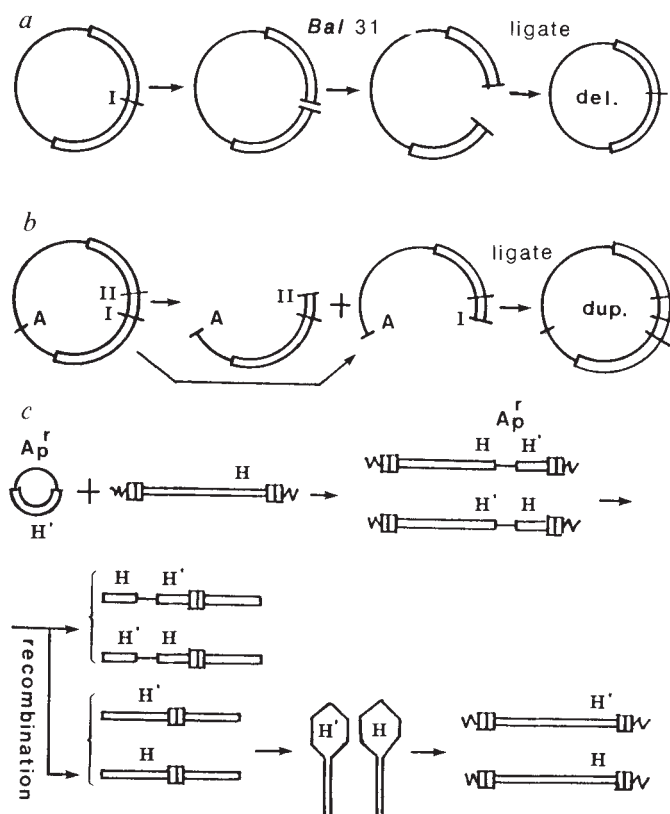


Fig. 1 Production of deletion (del.) and duplication (dup.) mutants in gene *H* of λ phage by *in vitro* mutagenesis. *a*, Production of deletions in cloned gene *H*. The *Bcl*I fragment (base numbers 9,361-13,820⁹) of λ DNA containing gene *H* was inserted into the *Bam*HI site of pBR322 (ref. 3), where the direction of the λ sequence in the hybrid plasmid was counter-clockwise on the conventional map of pBR322. Then it was cleaved at the unique *Hpa*I site (I in the figure), digested to various extents with *Bal*31 exonuclease, and self-ligated with T4 DNA ligase. *b*, Production of a duplication in cloned gene *H*. The 5.5-kilobase (kb) fragment from the same hybrid plasmid cleaved with *Hpa*I (I) and *Ava*I (A) and the 3.8-kb fragment from cleavage with *Pvu*II (II) were purified by electrophoresis in an 0.7% agarose gel and joined together with T4 DNA ligase. *c*, Introduction of cloned gene *H* having a deletion or duplication mutation (*H'*) into the λ genome. Cells of *E. coli* HI97 *polA supF* (λ clts857 Sam7) were transformed with the plasmids shown in *a* and *b* using selection for ampicillin resistance (*Ap*^r). (They were plated with tryptone top agar on LA plates with magnesium⁵ containing also 50 μ g ml⁻¹ ampicillin and incubated for 24 h at 32 °C.) Because these plasmids, having the replication origin of ColE1³, cannot replicate in *E. coli* *polA* (ref. 11), the transformants were those in which the plasmids were inserted by homologous recombination into gene *H* of the λ prophage (right-hand top row of *c*). The efficiency of transformation was about 10 colonies per μ g DNA. The ampicillin-resistant lysogens were cultured in M9A medium⁵ and subjected to heat-induction of the prophage. The λ DNA having the plasmid insertions (at top left in the second row of *c*) was too long to be packaged into λ phage particles. The packaged DNA molecules were those from which the inserted plasmids had been excised by homologous recombination. Through insertion and excision the wild-type gene *H* of the original λ genome was replaced by the mutated gene *H* of the plasmids with a certain probability. Therefore, the lysates contained both wild-type and *H* deletion (or duplication) phages (bottom left on the second row of *c*). *E. coli* W3350 Su⁻ was lysogenized by these phages. Lysogens having the *H* deletion or *H* duplication prophage were distinguished from those having wild-type prophage by their inability to produce active phage after heat induction. It is not completely excluded that phages having a deletion or duplication in gene *H* may form plaques, but none of the phages of this study did so. (See ref. 12 for detailed methods of genetic engineering and ref. 5 for the test of defective prophage mutants.)

Table 1 Properties of *in vitro* deletion and duplication λ mutants

Mutant	Site*	Homology†	No. of tail disks‡	Phenotype
del 101	11,392-11,628 (Arg 285-Ala 363)	4	30	Fragile tail tip
del 102	11,120-11,890 (Ala 194-Thr 450)	1	26	
del 103	11,226-11,810 (Lys 229-Asn 423)	3	27	
del 104	11,136-11,738 (Glu 199-Glu 399)	5	26	Defective in DNA injection
del 105	11,507-11,611 (Glu 323-Trp 357)	3	31	
del 106	11,070-12,005 (Ala 177-His 488)	2	21	
del 107	11,330-11,587 (Asp 264-Asn 349)	9	30	
del 108	11,196-11,822 (Glu 219-Lys 427)	2	26	
del 109	11,231-11,887 (Thr 231-Ala 449)	3	25	
del 110	11,382-11,822 (Asp 281-Lys 427)	2	27	
del 111	10,650-12,224 (Ala 37-Gln 561)	3	13	
del 112	11,079-12,743 (Thr 180-Ala 734)	2	12	
dup 1	11,586-12,101 (Asn 349-Gln 520)	0	39	

* Nucleotide positions (see ref. 9 for numbering of bases in the λ genome) of deleted or duplicated bases (amino-acid residues of gpH in parentheses) as determined by DNA sequencing (dideoxy method¹⁰) for the deletions and estimated by the method of construction (Fig. 1b) for the duplication.

† Number of homologous bases between the two ends of the deletion or duplication according to the DNA sequences. The deletion mutants have between one and nine identical bases at the two ends of the deletion. Such mutants were selected for, probably because ligation was performed without removing or filling in single-stranded ends generated by the *Bal*31 digestion. This might have enriched for those in-frame deletion mutants which have similar amino-acid sequences at the two deletion ends. However, the effect should not be large enough to influence the conclusion that defective phage particles are produced by most in-frame deletion mutants in the middle part of gene *H*.

‡ The number of disks in the tail tube as counted on electron micrographs of phage particles. The most frequent number among ten or more tails is shown. It agrees with the number estimated from the length measurements to within experimental error (± 1.5 disks).

or a duplication of 516 bases was introduced into the cloned gene *H* as described in Fig. 1a and b. Finally, the corresponding mutant λ phage were obtained by insertion of the plasmids into gene *H* of the λ genome followed by excision of the inserted plasmids (Fig. 1c). None of the mutants obtained in this way produces active phage particles.

Thirty deletion mutants and one duplication mutant were obtained in all. Although no selection was performed for mutants producing phage particles, the duplication mutant and twelve of the deletion mutants were found by electron microscopy to produce phage particles, among which tail length varies from mutant to mutant but is exactly determined for each mutant (Fig. 2). Only the remaining eighteen deletions, which probably cause frame-shifts in gene *H*, block tail assembly. This shows that the middle part of gpH is not important for other tail assembly and length determination functions and that it acts only as a spacer for the length measurement.

The phage particles produced by these deletion and duplication mutants cannot form plaques owing to various defects. Two deletion phage particles (del 101 and del 102) were found to have no tail tips and to have ejected DNA when lysates were observed in the electron microscope. Ten deletion phages and one duplication phage seem to be defective in DNA injection, as observed under the electron microscope after mixing with *Escherichia coli* cells. Scandella and Arber⁴ and Katsura⁵ had already shown that some mutants (probably point mutants) in the middle part of gene *H* have altered properties in DNA injection. Two plaque-forming mutants with a deletion in the middle part of gene *H*, reported by Katsura and Hendrix² are

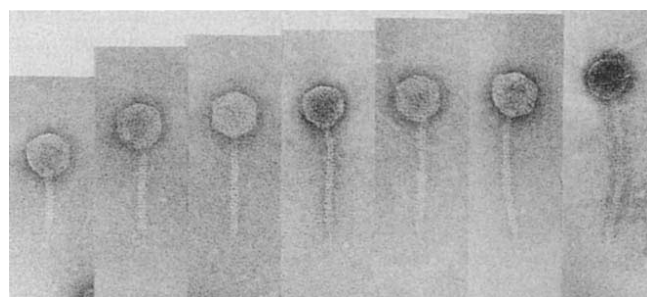


Fig. 2 Electron micrographs of phage particles of various tail lengths. From left to right: del 111 (number of tail-disks, 13), del 106 (21), del 109 (25), del 108 (26), del 107 (30), wild type (32) and dup 1 (39) (see ref. 13 for the definition of the disks in the tail tube). Phage particles were purified by CsCl step-gradient centrifugation. The micrographs were taken with a Hitachi HU-11B electron microscope at an acceleration voltage of 75 kV. Negative staining with 2% uranyl acetate. Magnification, $\times 105,000$.

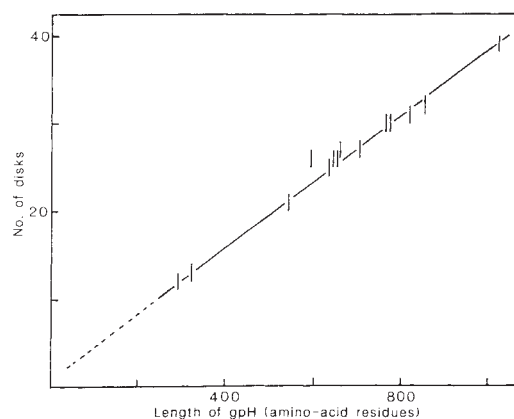


Fig. 3 Relation between the number of amino-acid residues of gpH and the number of tail disks in various phage particles. The number of amino-acid residues was calculated from the DNA sequence and the number of tail-disks was counted on electron micrographs as described in Table 1 legend. The length of the vertical bars in the figure shows the experimental error which is estimated to be ± 1.5 disks.

slightly defective in DNA injection, although they were selected for plaque-forming ability. These results show that the middle part of gpH is important in DNA injection and the stability of the tail tip. It is not unlikely that the fragility of the tail tip is due to aberrant reaction in DNA injection. However, it remains to be determined how exactly the structure of gpH is related to its function in DNA injection and especially how the middle part of gpH, which is not considered to be near to either the head or the tail tip, can influence DNA injection.

The positions of the twelve deletion mutations that allow formation of defective phage particles were determined by DNA sequencing (Table 1). As expected, they are all in-frame. As much as 65% (1,665/2,559 bases in del 112) of gene *H* could be deleted without losing the functions of tail assembly and length determination. The amino-acid residues deleted in the twelve mutants include Ala 37 to Ala 734 of the 853 amino-acid residues of gpH. The number of disks in the tail tube of the mutant phages was counted on electron micrographs (Table 1). It is approximately proportional to the number of amino-acid residues in gpH (Fig. 3). This means that nearly the whole molecule of gpH is used as a ruler for the measurement of tail length and that the middle part of gpH has a relatively uniform

structure with respect to length per amino-acid residue (about 1.6 Å per residue). This is consistent with the secondary structure prediction⁶ of gpH: mainly (about 60%) α -helical, where the distribution of the α -helices is relatively uniform except for the carboxy-terminal part which was not deleted in this study. It is known that gpH which has a relative molecular mass of 92,000 (M_r 92 K) is cleaved into gpH* (80 K) during tail assembly⁷. Because the cleavage seems to occur later than determination of the length of the tail⁸, this does not contradict the above conclusion.

Thus the method presented here is capable of changing the tail length of bacteriophage λ in a predictable fashion by *in vitro* mutagenesis, although these alterations lead to loss of biological activity. It has clarified the structure-function relationship of the ruler protein gpH and shows the feasibility of manipulating the size of supramolecular structures precisely, which may have many useful applications.

I thank Professor K. Takahashi for support. This work was supported by grants from the Ministry of Education, Science and Culture of Japan.

Received 8 January; accepted 16 March 1987.

1. Ulmer, K. M. *Science* **219**, 666-671 (1983).
2. Katsura, I. & Hendrix, R. W. *Cell* **39**, 691-698 (1984).
3. Bolivar, F. *et al.* *Gene* **2**, 95-113 (1977).
4. Scandella, D. & Arber, W. *Virology* **69**, 206-215 (1976).
5. Katsura, I. *Molec. gen. Genet.* **148**, 31-42 (1976).
6. Chou, P. Y. & Fasman, G. D. *A. Rev. Biochem.* **47**, 251-276 (1978).
7. Hendrix, R. W. & Casjens, S. R. *Virology* **61**, 156-159 (1974).
8. Tsui, L.-C. & Hendrix, R. W. *Virology* **125**, 257-264 (1983).
9. Sanger, F., Coulson, A. R., Hong, G. F., Hill, D. F. & Petersen, G. B. *J. molec. Biol.* **162**, 729-733 (1982).
10. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
11. Kingsburgh, D. T. & Helinski, D. R. *J. Bact.* **114**, 1116-1124 (1973).
12. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
13. Katsura, I. in *Lambda II* (eds Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. A.) 331-346 (Cold Spring Harbor Laboratory, New York, 1983).

An extrachromosomal factor causing loss of paternal chromosomes

John H. Werren, Uzi Nur & Danna Eickbush

Biology Department, University of Rochester, Rochester, New York 14627, USA

Extrachromosomal inheritance is ubiquitous among plants and animals; however, most extrachromosomal factors are uniparentally inherited through females, but not through males¹. Examples include chloroplasts, mitochondria and a variety of intracellular symbionts. The only known exception to maternal extrachromosomal inheritance in an animal is a paternally transmitted sex ratio factor (*psr*) which causes all-male families in the parasitic wasp, *Nasonia vitripennis*^{2,3}. Normally in this wasp, male offspring are haploid and develop from unfertilized eggs whereas females are diploid and develop from fertilized eggs. The *psr* factor is either a venereally transmitted infection which prevents egg fertilization (and therefore causes all-male families), or a factor transmitted to eggs by the sperm of males carrying *psr*, which somehow prevents incorporation of the paternal chromosomes. Here we report that sperm from *psr* males fertilizes eggs, but that the paternal chromosomes are subsequently condensed into a chromatin mass before the first mitotic division of the egg and do not participate in further divisions. Resulting haploid offspring are male, but have inherited the paternal factor. This extrachromosomal factor promotes its own transmission at the expense of the paternal chromosomes, and therefore can be considered a 'selfish' genetic element.

Table 1 Presence of sperm and dense chromatin mass in eggs fertilized by males of various types

0-5 minutes	Sperm	
	found	not found
<i>psr</i>	23	4
Control	8	1
Non- <i>psr</i>	12	1

45-85 minutes	Mitotic chromosomes		not visible
	visible	Mass	
	Mass present	absent	
<i>psr</i>	23	2	52
Control	0	21	30
Non- <i>psr</i>	2	24	24

Top: the presence or absence of visible sperm in eggs 0-5 min after oviposition was recorded. Sperm are readily seen in all three groups. Bottom: eggs with visible mitotic chromosomes were scored for the presence or absence of the dense chromatin mass. This mass typically occurs in *psr* fertilized eggs, but never in control eggs. Non-*psr* ('revertant') fertilized eggs occasionally show condensation of the paternal chromatin.

Nasonia vitripennis is a small (~3 mm) wasp which parasitizes the pupae of various fly species. The female wasp typically lays 20-40 eggs into the host and offspring take approximately 14 days to develop at 25 °C. As a result of haplodiploidy (females developing from fertilized eggs and males developing from unfertilized eggs), this wasp can control the sex ratio among its offspring by controlling sperm access to eggs. The wasp actively manipulates the sex ratio among offspring in a pattern which generally conforms to local mate competition theory⁴⁻⁷. In addition to the facultative sex-ratio control exercised by 'normal' wasps, an assemblage of three extrachromosomal elements that can distort the sex ratio also occur in *N. vitripennis*. One is maternally transmitted and causes a female-biased sex ratio⁸. A second is both maternally and infectiously transmitted and kills unfertilized eggs but not fertilized eggs^{9,10}, and the third (*psr*) is paternally transmitted and causes all-male families^{2,3}. Up to a quarter of wasps in natural populations carry at least one of these factors¹¹. The *psr* factor is of special interest because it is the only documented case of paternal extrachromosomal inheritance among animals. Paternal extrachromosomal inheritance of chloroplasts is known in some plants¹².

We conducted a cytogenetic study of the events following egg oviposition to determine whether *psr* sperm fertilize the eggs and, if so, what happens to the paternal chromosomes. Cytological procedures were similar to those of Ryan and Saul¹³. Eggs were fixed in Carnoy's fixative between 0 and 150 min after oviposition by the wasp. One to three days after fixation, eggs were individually stripped of their chorion and stained in 2% acetolactic orcein. These were then examined for the presence of spermatozoa or chromosomes. Not all males from a *psr* brood inherit the trait and those which do not are believed to be derived from unfertilized eggs³. Females mated to males from *psr* broods were given a second batch of hosts to determine whether the males with whom they mated expressed the *psr* (all-male family) trait. Thus, three types of fertilized eggs were examined, *psr* fertilized eggs, non-*psr* fertilized eggs (by males from *psr* families which did not themselves express the *psr* trait) and control fertilized eggs.

Sperm can easily be seen in eggs fertilized by *psr*, non-*psr* and control males within the first 5 min after oviposition (Table 1, top part). Thus sperm from *psr* males does fertilize eggs. But by the first mitotic division, a dramatic difference exists between *psr* and control fertilized eggs. A normal diploid complement is typically present in control eggs, but in *psr* fertilized eggs, approximately half the chromosomes clump into a dense

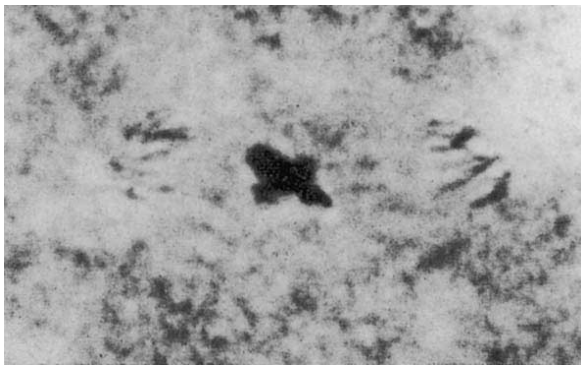


Fig. 1 The appearance of the dense chromatin mass of paternal chromosomes at the second mitotic division in a *psr* fertilized egg. Haploid sets of maternal chromosomes are seen in anaphase. The paternal chromosomes do not participate in the mitotic division.

chromatin mass, and do not participate in the mitotic division (Fig. 1).

During interphase, mitotic chromosomes are not visible (with acetolactic-orcein staining) in any of the experimental groups. The chromatin mass in *psr* fertilized eggs is also not visible during this time. However, during the second mitotic (cleavage) division, both chromosomes and the chromatin mass are again visible. There is invariably only one chromatin mass in *psr* eggs, which is typically associated with one of the dividing chromosome groups. The chromatin mass appears to be disintegrating by the sixth cleavage division. We do not know whether DNA from the mass undergoes any replication, but developing offspring do remain haploid and develop into males. Among those eggs in which the 1st or 2nd cleavage chromosomes are visible (in prophase to telophase), the chromatin mass is present in 23/25 *psr* eggs, 0/21 control eggs and 2/24 non-*psr* eggs (Table 1, bottom part). Clearly, occurrence of the chromatin mass is associated with *psr* eggs and not with controls (Fisher's exact test, $P < 0.001$). The chromatin mass does occur in some of the non-*psr* eggs. This suggests occasional incomplete expression of this trait in eggs fertilized by apparently revertant males.

It was not possible to determine by cytological observation whether maternal or paternal chromosomes form the condensed mass, but this can be demonstrated by genetic crosses. *N. vitripennis* has five chromosomes and mutant marker stocks are available for each of the five linkage groups¹⁴. Table 2 shows

Table 2 Results of crosses between *psr* males (with wild-type genotype) and mutant marker females for each linkage group

Linkage group	Mutant marker	Male offspring with:	
		maternal genotype	paternal genotype
I	<i>pe</i> 333, <i>pu</i>	884	0
II	<i>bk</i> 424	1,259	0
III	<i>pe</i> 100	1,300	0
IV	<i>st</i> 473	878	0
V	<i>st</i> 318	1,427	1

the results of crossing otherwise wild-type *psr* males to a mutant marker stock for each of the linkage groups. For each group, the mutant marker was represented in the resulting male offspring, showing that the maternal chromosome was transmitted but that the paternal chromosome was not. Previous studies had only confirmed this pattern for one of the linkage groups². Test confirmed that the males carried the *psr* trait, demonstrating its extrachromosomal inheritance. The linkage group V cross had a single paternal genotype out of 1,427 progeny scored, which is likely to represent a back mutation.

We conclude that sperm from *psr* males fertilize eggs and that paternal chromosomes are condensed before the first mitotic division of the zygote. Genetic studies show that it is the paternal chromosomes which are lost and that transmission of *psr* is strongly correlated with egg fertilization by sperm³. The *psr*

factor is therefore transmitted to the egg either in or on the sperm.

Paternal genome loss has been described in some scale insects, mites and sciarid flies^{15,16}. However, in these systems such loss is a normal mode of sex determination rather than a result of a paternally inherited extrachromosomal factor. Paternal genome loss also occurs in crosses between cytoplasmically incompatible strains of *N. vitripennis*¹³. Such cytoplasmic incompatibility is maternally inherited in the egg cytoplasm, unlike the paternally inherited *psr* factor. In addition, the paternal chromosomes in incompatible crosses form a diffuse tangled mass at the first cleavage division, rather than the dense chromatin mass observed with *psr*. The molecular mechanisms of paternal genome loss is unknown for any of these systems.

We do not know what causes the effects of *psr*. The *psr* factor may be a microorganism incorporated onto or in the sperm. One study has shown that certain viruses in fish readily adhere to sperm surfaces¹⁷. Alternatively, *psr* may be a retrovirus or transposon which is temporarily incorporated into the paternal genome. Such an agent would have to transfer to the maternal genome in the egg after fertilization, because the paternal genome is subsequently lost. We are now studying these possibilities.

The *psr* factor may not be a gene in the traditional sense, but it can clearly be considered an extremely 'selfish' genetic element¹⁸⁻²⁰. Because *psr* is paternally inherited, the production of all-male families greatly enhances its transmission to future generations. This is accomplished at the cost of the paternal chromosomes, which are condensed into a mass and are not inherited. The maternal chromosomal genome suffers the same fate in the next generation because it is present in *psr* males. As a result, chromosomal genes should be strongly selected to suppress expression of the *psr* factor, because it imposes such a strong selective cost on the rest of the genome.

We thank Bruce Grant and George Saul for some of the stocks used in this study and Tom Eickbush and Jeanne Peterson for helpful comments on the manuscript. This research was supported by Grants from the NSF.

Received 28 November 1986; accepted 27 February 1987.

1. Birky, C. W. Jr *Science* **222**, 468-475 (1983).
2. Werren, J. H., Skinner, S. W. & Charnov, E. L. *Nature* **293**, 467-468 (1981).
3. Werren, J. H. & van den Assem, J. *Genetics* **114**, 217-233 (1986).
4. Wylie, H. G. *Can. Ent.* **98**, 645-653 (1966).
5. Werren, J. H. *Evolution* **37**, 116-124 (1983).
6. Charnov, E. L. *The Theory of Sex Allocation* (Princeton University Press, 1982).
7. Werren, J. H. *Science* **208**, 1157-1160 (1980).
8. Skinner, S. W. *Science* **215**, 1133-1134 (1982).
9. Skinner, S. W. *Genetics* **109**, 745-754 (1985).
10. Werren, J. H., Skinner, S. W. & Huger, A. M. *Science* **231**, 990-993 (1986).
11. Skinner, S. W. thesis, Univ. Utah (1983).
12. Sears, B. B. *Stadler Symp.* **15**, 119-138 (1983).
13. Ryan, S. L. & Saul, H. G. B. *Molec. gen. Genet.* **103**, 24-36 (1968).
14. Saul, G. B., Whiting, P. W., Saul, S. W. & Heidner, C. A. *Genetics* **52**, 1317-1327 (1965).
15. Nur, U. R. *Ent. Soc. Lond. Symp.* **10**, 97-117 (1980).
16. Bull, J. J. *The Evolution of Sex Determining Mechanisms* (Benjamin, Menlo Park, California, 1983).
17. Mulcahy, D. & Pascho, R. J. *Science* **225**, 333-335 (1984).
18. Dawkins, R. *The Selfish Gene* (Oxford University Press, 1976).
19. Doolittle, W. F. & Sapienza, C. *Nature* **284**, 601-603 (1980).
20. Orgel, L. E. & Crick, F. H. C. *Nature* **284**, 604-607 (1980).

Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues

Y. Kuchino*, F. Mori*, H. Kasai*, H. Inoue†, S. Iwai†, K. Miura†, E. Ohtsuka† & S. Nishimura*

* Biology Division, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Japan

† Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

It has been shown previously that deoxyguanosine residues in DNA are hydroxylated at the C-8 position both *in vitro* and *in vivo* to produce 8-hydroxydeoxyguanosine (8-OH-dG) by various agents that produce oxygen radicals such as reducing reagents-O₂, metal ions-O₂, polyphenol-H₂O₂-Fe³⁺, asbestos-H₂O₂ or ionizing radiation¹⁻⁵. These agents are mostly either mutagenic or carcinogenic; therefore, the formation of 8-OH-dG can also be considered a likely cause of mutation or carcinogenesis by oxygen radicals. It is of interest to know whether the 8-OH-dG residue in DNA is misread during DNA replication. To answer this question, we have examined the effect of the 8-OH-dG residue in DNA on the fidelity of DNA replication using a DNA synthesis system *in vitro* with *Escherichia coli* DNA polymerase I (Klenow fragment). The synthetic oligodeoxynucleotides, with or without an 8-OH-dG residue in a specified position, were chemically synthesized and used as templates for DNA synthesis under the conditions of the dideoxy chain termination sequencing method. Surprisingly, in addition to misreading of the 8-OH-dG residue itself, pyrimidines next to the 8-OH-dG residue (G has not yet been tested) were also misread.

Figure 1 shows the nucleotide sequences of two synthetic 8-OH-dG-containing oligodeoxynucleotides (fragments B and D) and their counterparts with normal G in place of 8-OH-dG (fragments A and C) and gives details of their chemical synthesis. Figure 2 shows an example of the base composition and sequence analysis of one of the chemically synthesized oligodeoxynucleotides (fragment D). HPLC analysis of the deoxynucleoside mixture, produced from fragment D by nuclease P₁ digestion followed by *E. coli* alkaline phosphomonoesterase, showed that the 8-OH-dG content was more than 90% of the theoretically expected value. The sequence of fragment D was found to be homogeneous, as indicated by wandering-spot analysis.

Figure 3 shows the autoradiograms of sequencing gels of products of the dideoxy chain termination method using the chemically synthesized oligodeoxynucleotides as templates. In the case of oligodeoxynucleotide B, in which 8-OH-dG is positioned between T and C, the 8-OH-dG residue directed the insertion of A, T, C or G with an almost equal frequency, indicating that 8-OH-dG completely lacks specific base-pairing. It was striking that the deoxycytidine residue adjacent to the 3'-side of the 8-OH-dG also directed insertion of all four residues. In addition, the thymidine residue on the 5'-side of the 8-OH-dG directed the insertion of C and T to a small extent in addition to its normal base pairing with A. Note that the control oligodeoxynucleotide (fragment A), with a normal G in place of the 8-OH-dG, was faithfully reproduced without any misreading, when DNA synthesis was carried out under the same conditions as for fragment B. This indicates that the 8-OH-dG residue has an overwhelming effect on template-directed DNA synthesis in that it causes misreplication not only at the position of the 8-OH-dG residue itself, but also at neighbouring bases. The misreading caused by the presence of the 8-OH-dG residue was also observed in fragment D, in which the 8-OH-dG was between A and T (Fig. 3a). In this case, however, the 8-OH-dG residue itself directed the insertion of only T in addition to the correct C, although the T on the 3'-side

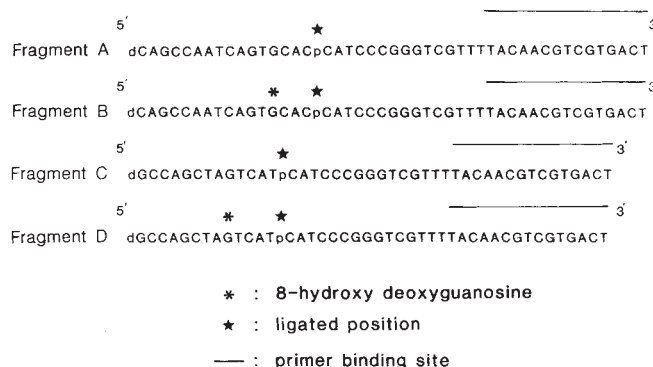


Fig. 1 Sequence of four chemically synthesized oligodeoxynucleotides, with or without 8-OH-dG used as templates for DNA synthesis. ★, Position of ligation; *, 8-OH-dG base.

Methods. The oligodeoxynucleotides (fragments A, B, C and D) were prepared by joining 5' 13-mers or 16-mers to the 3' 30-mer using a complementary 15-mer (5'-GGGATGATGACTAGC-3') as a splint. The oligodeoxynucleotides without 8-OH-dG residue were synthesized by the phosphoramidite method¹³, using a DNA synthesizer (Applied Biosystems 380A). The 8-OH-dG 13-mer and 16-mer were synthesized by the solid-phase phosphotriester method using protected dimers and a monomer¹⁴; N²-acetyl-5'-monomethoxytrityl-8-methoxydeoxyguanosine 3'-(O-chlorophenyl) phosphate was used as the monomer unit. The 8-methoxydeoxyguanosine was prepared from deoxyguanosine by a modification of the reported procedures¹⁵, and the 8-O-methyl group was removed in the last stage of the oligomer synthesis. For example, the 13-mer, used for the preparation of fragment D, was synthesized starting with thymidine resin (5 μmol)¹⁶. After condensation and cleavage from its support, the product was heated in concentrated ammonia at 55 °C for 2.5 h and concentrated. The 13-mer-containing 8-methoxydeoxyguanosine (5.5 A₂₆₀ units), which was found to be homogeneous by HPLC analysis, was dissolved in water (0.1 ml) and treated with N,N'-dimethylformamide (0.4 ml) and triethylamine (0.1 ml) at 35 °C for 24 h under N₂. The mixture was partitioned between water and chloroform and the aqueous phase evaporated with added water, and washed with ethyl acetate. The completely deblocked 13-mer (2.6 A₂₆₀ units) was separated by reversed-phase (RP) HPLC from the methoxy compound, and shown to be homogeneous by RP and anion exchange HPLC. For the enzymatic joining, the 30-mer (4 A₂₆₀ units) was phosphorylated by T4 polynucleotide kinase and ATP. Half the reaction mixture was annealed with the 13-mer (1.0 A₂₆₀ unit) and 15-mer (1.0 A₂₆₀ unit). After the addition of 2-mercaptoethanol, the mixture was treated with T4 DNA ligase (700 units) at 15 °C for 18 h in a total volume of 0.6 ml. The product, after successive treatments with phenol and ether, was dissolved in 90% formamide and separated by electrophoresis using a 10% polyacrylamide gel (2 mm thick) containing 8 M urea. The fragment (D) (1.2 A₂₆₀ units) was then eluted from the gel.

of the 8-OH-dG directed the insertion of all four bases. However, the A located at the 5'-side of the 8-OH-dG was correctly read, directing the insertion of T only. Thus it would appear that the extent of the misreading due to 8-OH-dG depends on the nucleotide sequences which surround the 8-OH-dG residue.

When the DNA synthesis was carried out *in vitro* without the addition of dideoxynucleoside triphosphates, almost no termination was detected in the region of the 8-OH-dG residue (Fig. 3b). Therefore, the random termination observed in the position of the 8-OH-dG and its neighbouring bases, in the sequencing gels described above, must be due to the insertion of a dideoxynucleotide and not to the mere termination of DNA synthesis, as has been reported previously for other DNA adducts⁶⁻⁹. Furthermore, the rate of the DNA synthesis using 8-OH-dG-containing oligodeoxynucleotides as templates, was about equal to that using control oligodeoxynucleotides without 8-OH-dG (data not shown). To exclude the possibility that such misreading only occurs when dideoxynucleoside triphosphates are

Fig. 2 Analysis of the base composition (a) and the nucleotide sequence (b) of one of the chemically synthesized oligodeoxynucleotides (fragment D).

Methods. In a, fragment D (0.1 A_{260} unit) was dissolved in 50 μ l of 20 mM sodium acetate buffer (pH 4.8), digested with 2 μ g of nuclease P_1 at 37 °C for 30 min and then treated with 0.1 unit of *E. coli* alkaline phosphomonoesterase in Tris-HCl buffer (pH 7.5) for 1 h. The resulting deoxynucleoside mixture was analysed by HPLC (column, Beckman Ultrasphere ODS, 0.46 $\times$ 25 cm; eluent, 10% aqueous methanol containing 12.5 mM citric acid/25 mM sodium acetate/30 mM NaOH/10 mM acetic acid; flow rate, 1 ml min⁻¹; electrochemical (EC) detection with 600 mV (oxidation)). The molar ratio of 8-OH-dG to dG in the sample was 0.10, as judged from the peak height of authentic 8-OH-dG with the EC detector and the peak height of dG with the UV detector. In b, fragment D, labelled with ³²P at the 5' end by polynucleotide kinase in the presence of [α -³²P]ATP was partially digested with nuclease P_1 , according to the procedures described by Silberklang *et al.*¹⁷. DNA digests were fractionated by two-dimensional thin-layer chromatography using homomixes prepared by the method of Jay *et al.*¹⁸.

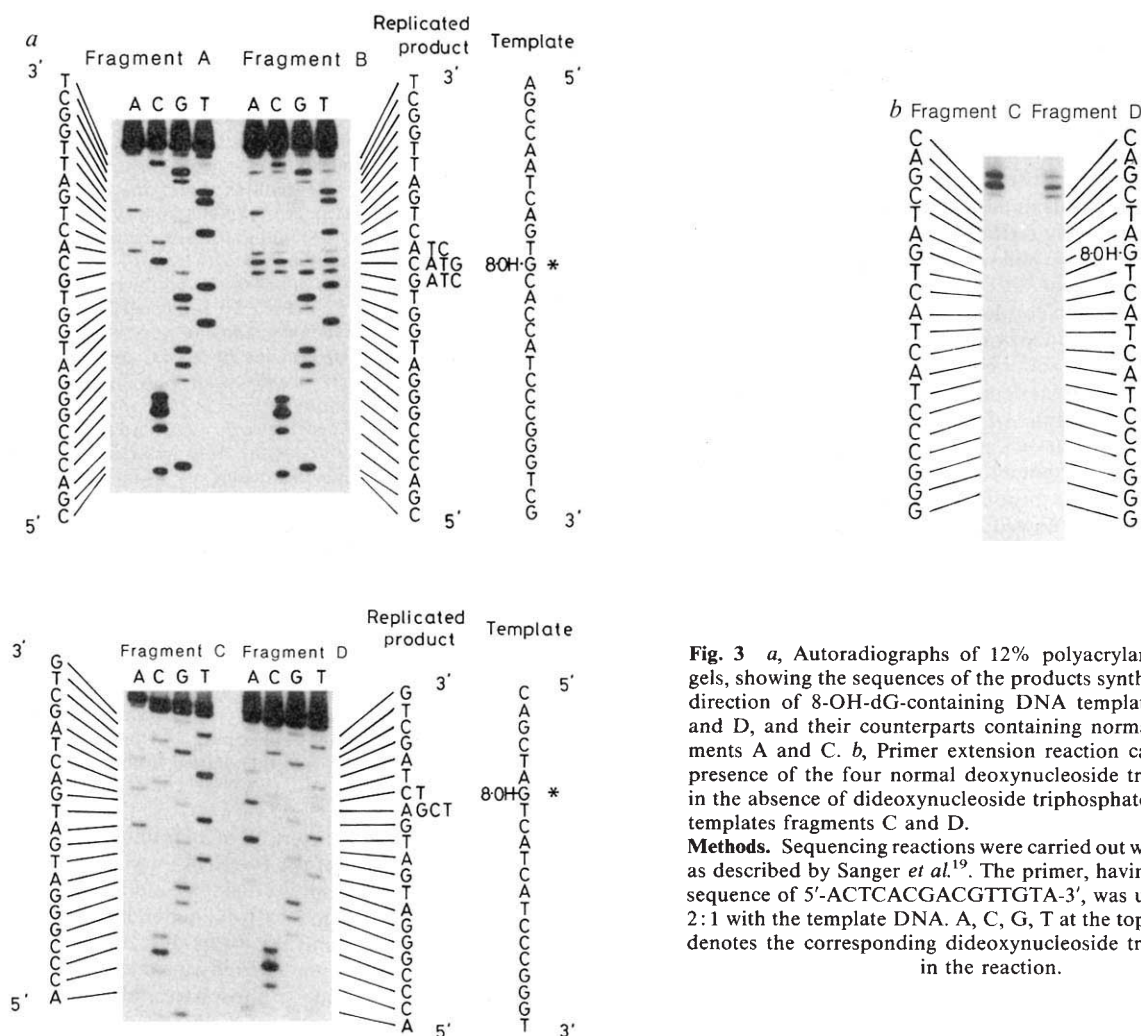
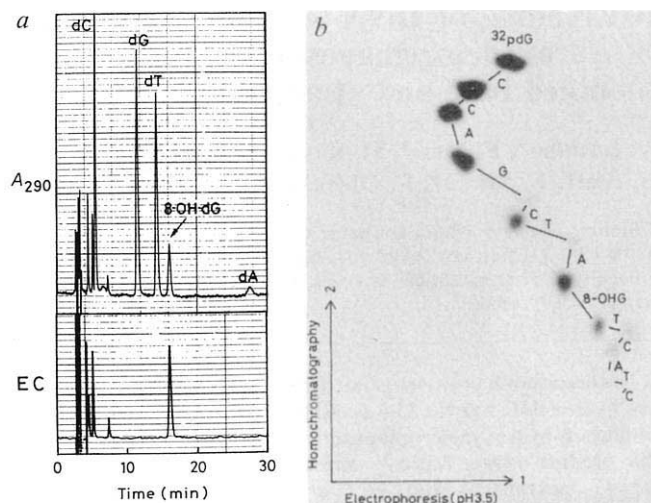


Fig. 3 a, Autoradiographs of 12% polyacrylamide sequencing gels, showing the sequences of the products synthesized under the direction of 8-OH-dG-containing DNA templates, fragments B and D, and their counterparts containing normal guanine, fragments A and C. b, Primer extension reaction carried out in the presence of the four normal deoxynucleoside triphosphates, but in the absence of dideoxynucleoside triphosphates using as DNA templates fragments C and D.

Methods. Sequencing reactions were carried out with [α -³²P]dATP as described by Sanger *et al.*¹⁹. The primer, having the nucleotide sequence of 5'-ACTCACGACGTTGTA-3', was used in a ratio of 2:1 with the template DNA. A, C, G, T at the top of each column denotes the corresponding dideoxynucleoside triphosphate used in the reaction.

incorporated, and not during the incorporation of the normal deoxynucleoside triphosphates, fragments A and B were used as templates in the absence of dideoxynucleoside triphosphates. The primer used was 5'-³²P-labelled AGTCACGACTTGA and the newly synthesized DNA was analysed by the method of Maxam and Gilbert¹⁰. Although the misreading observed was not so clear, misreading in the region of 8-OH-dG and its adjacent bases was undoubtedly evident even in the absence of

dideoxynucleoside triphosphate incorporation (data not shown).

The experiments described here are only representative of the effects of 8-OH-dG on DNA replication *in vitro*. A conclusive answer to the question of whether or not the misreading observed *in vitro* is relevant *in vivo* could be obtained by recloning and sequencing M13 phages propagated *in vivo* from an 8-OH-dG-containing artificial phage.

Similar approaches to those reported in the present paper have previously been made in analysing the effects of the presence in DNA of thymine glycol⁹ or of the DNA-adducts produced by treatment with chemical carcinogens such as 4-hydroxyaminoquinoline 1-oxide⁸, acetylated 2-aminofluorene^{6,7} and deacetylated 2-aminofluorene⁶. In these instances, DNA lesions caused by modification of the bases only inhibited DNA elongation and no misreading was observed. O'Connor and Stohrer reported that oligodeoxynucleotides, having a site-specific guanine-8-aminofluorene adduct or its oxidative degradation product, are by-passed, either when the templates are read by a primer extension, or by a nick-filling procedure¹¹. But the DNAs used in these experiments were prepared, in most cases, by chemical modification, causing the modified base to be randomly located in the DNA instead of being in one specific position. Our approach, synthesizing chemically an oligodeoxynucleotide with the modified base in a specified position and then using it as a template in the dideoxy chain termination sequencing system, should provide clearer information on the effect of DNA adducts in DNA replication.

Although the molecular mechanism by which the 8-OH-dG affects the fidelity of DNA replication, is of great interest, nothing is known about it at present. X-ray crystallographic analysis of 9-ethyl-8-hydroxyguanine shows that the purine skeleton of the molecule in the crystal is in the 6,8-diketo form (unpublished results). It is likely, therefore, that in the native form of DNA the 8-OH-dG residue would be shifted mainly to its 8-keto form, with a consequent change in its base-pairing properties. It is also known that 8-OH-dG is prone to adopt a *syn* conformation (unpublished results). The effect of an added oxygen atom in 8-OH-dG will be quite different from that of other DNA adducts that have a bulky side chain in position 8 of the guanine residue.

It is considered probable that oxygen radicals are involved in mutation, carcinogenesis and ageing¹². Oxygen radicals are produced not only by exogenous chemicals or radiation, but also by intracellular biochemical metabolism. When irradiated mice were kept without further irradiation, the amount of 8-OH-dG in DNA was found to have decreased to a certain extent, indicating the presence of a repair mechanism for removing 8-OH-dG from the cells. Removal of the 8-OH-dG was not complete, however, and a residual amount remained for a long period after irradiation⁵. It is possible, therefore, that the formation, by oxygen radicals, of 8-OH-dG in DNA, is an important cause of mutation and carcinogenesis. In addition, loss of fidelity of DNA synthesis at positions next to the modified base, clearly shown here provides a possible new molecular mechanism of mutation.

This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare for a Comprehensive 10-Year Strategy for Cancer Control and a grant from the Ministry of Education, Science and Culture.

Crystallographic study of a β -lactam inhibitor complex with elastase at 1.84 Å resolution

Manuel A. Navia*, James P. Springer*, Tsau-Yen Lin*, Hollis R. Williams*, Raymond A. Firestone†, Judith M. Pisano†, James B. Doherty†, Paul E. Finke† & Karst Hoogsteen*

Departments of * Biophysics and † Membrane and Arthritis Research, Merck Sharp and Dohme Research Laboratories, PO Box 2000, Rahway, New Jersey 07065, USA

The continuing discovery and development of β -lactams as antibiotics has had an unparalleled impact on the overall health and well-being of society. Recently, appropriately substituted cephalosporins were shown to be potent inhibitors of elastase¹, suggesting a novel therapeutic role for the β -lactams in the control of emphysema and other degenerative diseases^{2,3}. We have now solved and partially refined at atomic resolution the structure of a complex of porcine pancreatic elastase with the time-dependent irreversible inhibitor 3-acetoxymethyl-7- α -chloro-3-cephem-4-carboxylate-1,1-dioxide tert-butyl ester (I), the most potent of the β -lactam elastase inhibitors yet reported¹. (Porcine pancreatic elastase is a close relative of the desired drug target, human polymorphonuclear leukocyte elastase.) A mechanism of action is presented, based on the structure and on biochemical evidence (T.-Y.L. *et al.*, in preparation), which clarifies the operational similarities and differences between β -lactam elastase inhibitors and antibiotics. Features of the reaction include the expulsion of a leaving group at the cephalosporin 3' position and the formation of two covalent bonds with the active site of porcine pancreatic elastase at residues Ser 195 and His 57.

A difference electron density map of the complex of compound I (see Fig. 1a) with porcine pancreatic elastase (PPE) at 1.84 Å resolution is shown in Fig. 1b. Apart from minor readjustments in the bound-water structure, the difference map is featureless, suggesting that the enzyme was unperturbed by inhibitor binding. The end-state structure shown is the best-fit of a series of possible models for the reaction. Alternative reaction product models led to clearly inconsistent or grossly incomplete fits to the electron density map, which by its high resolution and quality imposes severe structural constraints on the system. Figure 1c shows the same view of the complex in schematic form, labelled according to convention⁴.

An overview of the enzyme-inhibitor complex is shown in Fig. 2. We find Ser 195 OG covalently bound to C8 of the inhibitor by an ester bond. Electron density accommodates the O8 carbonyl oxygen of the inhibitor, which is within hydrogen bonding distance of the backbone amide nitrogens of Gly 193 and Ser 195. The β -lactam ring has been opened at the N5-C8 bond, and it was not possible to fit any closed-ring models into the electron density. Neither was density observed in the vicinity of C7 of I, indicating that the 7' Cl is absent. A double bond has been formed between C6 and C7; models involving tetrahedral carbon atoms at these positions lead to severe inconsistencies in fitting the remainder of the structure. The dihydrothiazine ring of the inhibitor remains intact in the complex, as suggested for the cephalosporin antibiotics (see for example, ref. 5). Ring opening between S1 and C5 (equivalent to C6 in the cephalosporins) is an integral part of proposed β -lactamase inhibition mechanisms involving penams, and penam sulphones⁶. As expected, the difference electron density at the sulphone position is the highest observed in the map. One of the sulphone oxygens is within hydrogen bonding distance of the side chain of Gln 192, and the other interacts with the backbone amide nitrogen of residue Val 216. The 3" acetoxy

Received 21 October 1986; accepted 26 January 1987.

1. Kasai, H. & Nishimura, S. *Nucleic Acids Res.* **12**, 2137-2145 (1984).
2. Kasai, H. & Nishimura, S. *Gann* **75**, 565-566 (1984).
3. Kasai, H. & Nishimura, S. *Gann* **75**, 841-844 (1984).
4. Kasai, H., Tanooka, H. & Nishimura, S. *Gann* **75**, 1037-1039 (1984).
5. Kasai, H. *et al. Carcinogenesis* **7**, 1849-1851 (1986).
6. Moore, P. D., Bose, K. K., Rabkin, S. D. & Strauss, B. S. *Proc. natn. Acad. Sci. U.S.A.* **78**, 110-114 (1981).
7. Moore, P. D., Rabkin, S. D., Osborn, A. L., King, C. M. & Strauss, B. S. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7166-7170 (1982).
8. Yoshida, S., Koizumi, O., Suzuki, R. & Tada, M. *Cancer Res.* **44**, 1867-1870 (1984).
9. Rouet, P. & Essigmann, J. M. *Cancer Res.* **45**, 6113-6118 (1985).
10. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
11. O'Connor, D. & Stohrer, G. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2325-2329 (1985).
12. Ames, B. N. *Science* **204**, 587-593 (1979).
13. Caruthers, M. H. *Science* **230**, 281-285 (1985).
14. Ikehara, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 5956-5960 (1984).
15. Lin, T.-S., Cheng, J.-C., Ishiguro, K. & Sartorelli, A. C. *J. med. Chem.* **28**, 1194-1198 (1985).
16. Ito, H., Ike, Y., Ikuta, S. & Itakura, K. *Nucleic Acids Res.* **10**, 1755-1769 (1982).
17. Silberklang, M., Gillum, A. M. & RajBhandary, U. L. *Nucleic Acids Res.* **4**, 4091-4108 (1977).
18. Jay, E., Bambara, R., Padmanabhan, R. & Wu, R. *Nucleic Acids Res.* **1**, 331-353 (1974).
19. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).

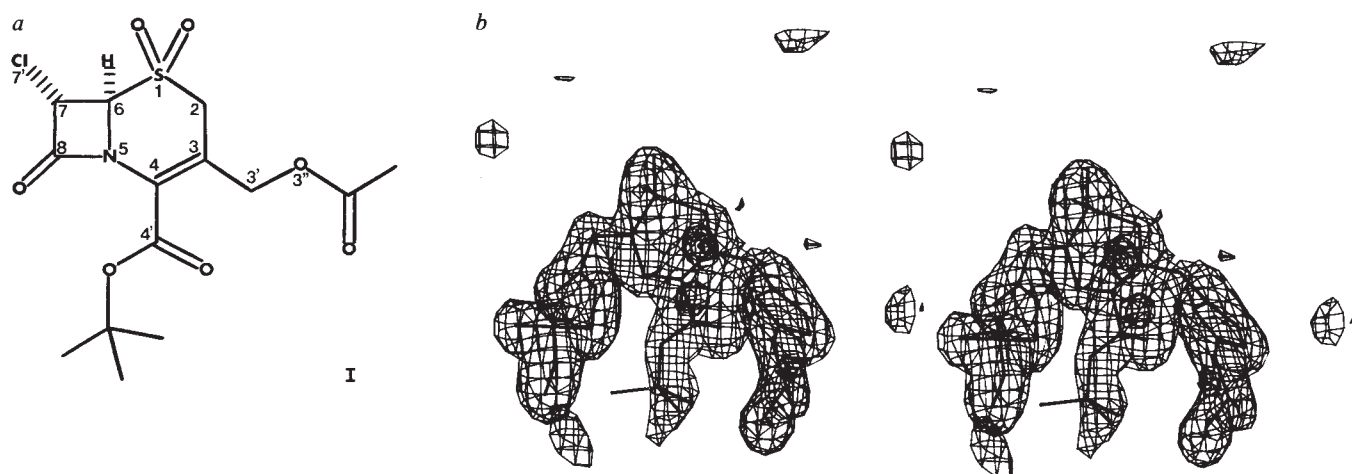


Fig. 1 *a*, Structure I, 3-acetoxymethyl-7- α -chloro-3-cephem-4-carboxylate-1,1 dioxido tert-butyl ester, compound XXII of Doherty *et al.*¹. *b*, Difference electron density map of the active site region of porcine pancreatic elastase in complex with the inhibitor I. The side chains of Ser 195 and His 57 were left out of the protein model used in phasing. The two residues are superimposed on the map, along with the best-fit model of I after its reaction with the enzyme. *c*, Schematic view of I in the PPE active site corresponding to *b* and labelled according to convention⁴.

Methods. PPE was isolated from a pancreatic extract (trypsin 1:300, US Biochemicals), as described¹⁸. Crystals were grown by vapour diffusion¹⁹, using published crystallization conditions¹⁸ which were adjusted from batch to batch in order to obtain crystals as large as a millimetre in length. Small macro-crystals were washed thoroughly and used as seeds, as described²⁰. Crystals were transferred into a 35% polyethylene glycol 6,000 (PEG) solution in 0.1 M sodium acetate at pH 5, equilibrated overnight, then soaked for a week in the same PEG solution saturated with inhibitor. X-ray data to 1.84-Å resolution were collected in shells from a single large crystal using a Syntex/Nicolet P2₁ diffractometer fitted with an extended (65 mm) arm and helium beam tunnel. A modified peak-top omega scan²¹ was used to measure reflection intensities at 0.5 to 2.0 degrees per min, depending on the resolution. Empirical absorption, linear radiation decay, and Lorentz-polarization corrections were applied^{22,23}. All reflections with intensity $I > \sigma(I)$ were included. Scaling, structure factor, and electron density calculations were done with the PROTEIN²⁴ structure analysis package. Starting phases for this study were obtained from a 1.84 Å structure of native PPE partially refined to an *R*-factor of 16.8% (unpublished work). To assist in the interpretation of the experimentally derived difference electron density of the complex of PPE with I, the structure of a close analogue, 3-methyl-7- α -methoxy-3-cephem-4-carboxylate-1,1-dioxido tert-butyl ester, was solved and refined to give an unweighted residual of 3.9%. Attempts to crystallize the inhibitor I were unsuccessful. Possible enzymatic modifications of I, based on the solved analogue, were constructed using the program MOLEDIT²⁵. These were fitted into the experimental difference electron density, using FRODO^{26,27} on an Evans and Sutherland Multi-Picture System.

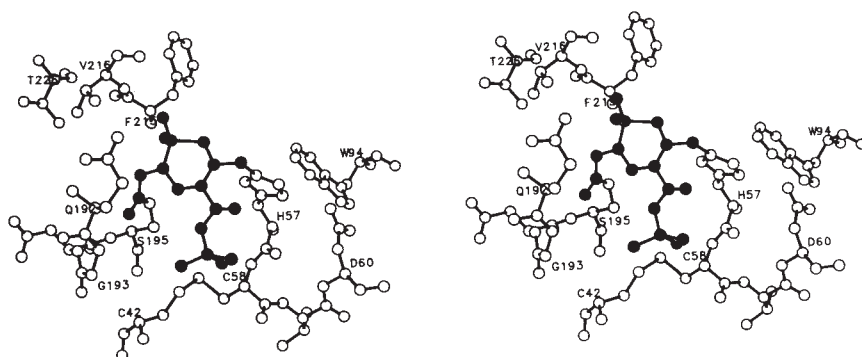
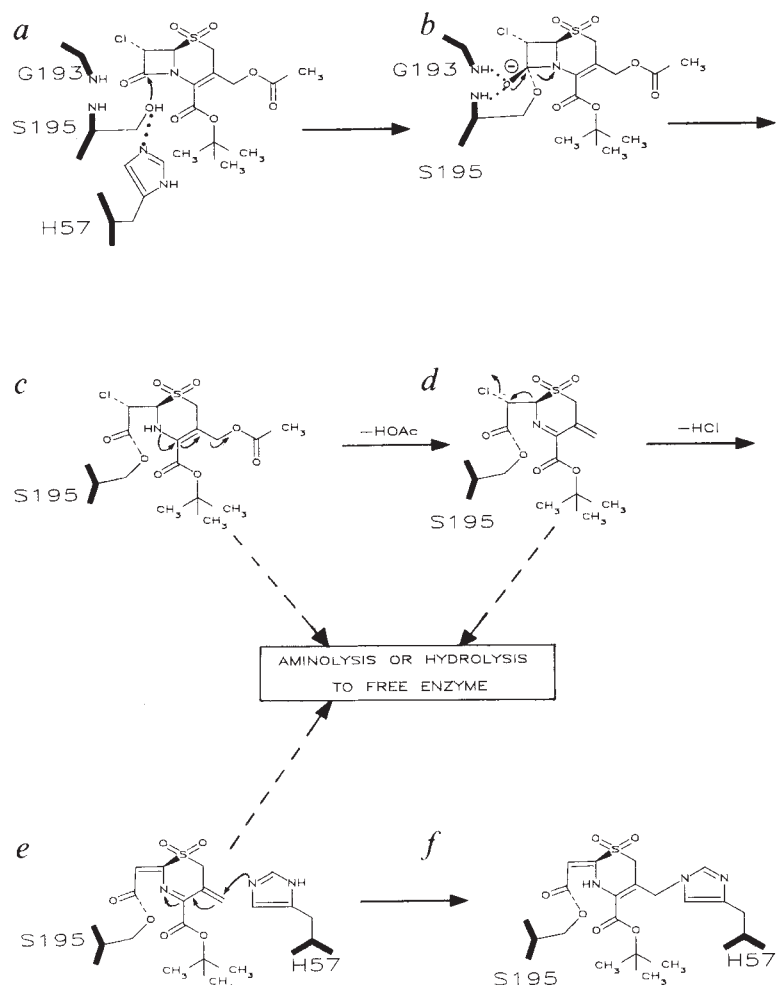


Fig. 2 Overall view of the active site region of the PPE complex with I, oriented as in Fig. 1. Open circles, atoms of the enzyme; a number of key residues (see text) are labelled at their α -carbons; Filled circles, inhibitor atoms.

substituent of I is absent in the complex, in agreement with proposals that require a good leaving group at this position for the antibiotics⁷. A second covalent bond is then formed between His 57 NE2 of PPE and C3' of I. To participate in this reaction, His 57 had to rotate away from its normal position in the catalytic triad⁸ with Ser 195 and Asp 102, where it was presumably involved in the initial acylation step. At its new position in the complex, His 57 ND1 is 2.9 Å from Asp 60 OD2, a distance comparable to that observed in the interaction of His 57 ND1 with Asp 102 OD1 in the catalytic triad (see for example Table 7 in ref. 9). In turn, Asp 60 OD1 is in contact with the indole ring of Trp 94, making the environment of Trp 94 sensitive to binding in the active-site region of the enzyme. This is consistent

with the observation of fluorescence quenching on inhibition (T.-Y.L. *et al.*, in preparation). Finally the 4-substituent tertiary butyl ester in the PPE-inhibitor complex fits at the edge of a large open area on the periphery of the enzyme (see Fig. 2). Its electron density tapers off somewhat away from the dihydrothiazine ring, suggesting increased mobility.

A consistent mechanism of action is presented in Fig. 3 involving an initial binding step, acylation by Ser 195, and nucleophilic attack by His 57 in the time-dependent irreversible inhibition of the enzyme. Elements of the initial binding of I to PPE can be inferred from Fig. 2, such as the hydrogen bonding of the sulphone oxygens of the inhibitor. It is also clear that the 7'- α -chloro substituent of the unreacted inhibitor originally



fitted into the S1 specificity pocket¹⁰ of PPE. Structure-activity correlations within the β -lactam elastase inhibitor series¹ strongly favour small 7'- α substituents. In the acylation reaction, the position of C8 and O8' of the inhibitor relative to the Ser 195 hydroxyl indicates an approach on attack from the α -face of the β -lactam ring. Binding of the β -lactam carbonyl oxygen O8 to the amide nitrogens of Gly 193 and (more weakly) Ser 195 can also be inferred, as well as stabilization of the tetrahedral intermediate on acylation, by analogy with what has been observed with L-amino-acid peptide inhibitors in other serine proteases (Table 6 in ref. 11).

A time-dependent loss of enzyme activity against substrate has been observed, as well as loss of inhibitory potential for compounds that act directly on Ser 195, such as diisopropyl-fluorophosphate and phenylmethanesulphonyl fluoride (T-Y.L. *et al.*, in preparation). Initial PPE inhibition by I is subject to reversal by hydroxylamine, probably by aminolysis of the bond between Ser 195 OG and inhibitor C8 that is observed in Fig. 3. With time (half-life $t_{1/2} = 2$ h), however, PPE inhibition becomes insensitive to hydroxylamine treatment. An exocyclic methylene group has been postulated at 3' after elimination of the leaving group at that position^{5,7}, and our scheme (Fig. 3d) proposes that this group is the target for nucleophilic attack by His 57. In fact, amino-acid analysis of acid hydrolysates of the complex made after 24 h reveals the loss of one out of the six histidine residues in the enzyme (unpublished data). The fact that this second reaction took place at the 3' position was unexpected, and could not have been easily determined other than by direct observation.

One requirement for activity shared by all β -lactam antibiotics is a negative charge at 4': a carboxylate in the cephalosporins or its equivalent in the other β -lactam antibiotic classes¹²⁻¹⁴. In contrast, the active β -lactam elastase inhibitors of Doherty *et al.*¹ have all been 4' esters or amides. The 4' free carboxylate analogues (for example, compound XLII in ref. 1) have not been effective inhibitors. We cannot explain this difference by any simple structural feature, such as steric interference by the enzyme, or the presence of a nearby repulsive charge (see Fig. 2). A consideration of possible initial inhibitor binding modes suggests that His 57 would be in close proximity to the negative charge of a 4' carboxylate analogue of I. Such a charge would strongly attract the protonated imidazole ring of His 57. Even at neutral pH, the polarizable imidazole might well be pulled away from its optimal position in the catalytic triad, preventing the initial acylation of inhibitor (Fig. 3a). Alternatively, the presence of a carboxylate substituent on the dihydrothiazine ring might adversely influence the development of negative charge needed for the expulsion of acetate at the 3' position (Fig. 3c). The importance of a good leaving group in the cephalosporin antibiotics has been stressed by a number of authors^{5,7}, and our structural results strongly support this view for the β -lactam elastase inhibitors as well. In addition, we provide indirect evidence for a postulated exocyclic methylene intermediate^{5,7}, subject to nucleophilic attack by His 57 (Fig. 3e).

Our favoured mechanism differs from those proposed for β -lactamase inhibition by penams and penam sulphones⁶, where acylation leads to ring opening and structural rearrangements before nucleophilic attack and irreversible inhibition. Cephalo-

Table 1 Preliminary restrained least squares refinement data for the porcine pancreatic elastase complex with I

	Target standard deviation	Initial model	Final refined model
R-factor*		33.2%	22.8%
Deviations from ideality†			
Bond distance (Å)	0.020	0.016	0.026
Angle distance (Å)	0.030	0.036	0.045
Planarity (Å)	0.040	0.051	0.055
Resolution range (Å)		10.0–2.5	10.0–1.84
No. of reflections		6,887	14,135
No. of atoms		1,841	1,913

The Hendrickson-Konnert program^{16,17} was used, as modified by E. Fluder for the IBM 3090.

* The R-factor is defined by the expression:

$$\Sigma ||F_o| - |F_c|| / \Sigma |F_c|$$

where $|F_o|$ are the observed structure factors and $|F_c|$ are the corresponding calculated structure factors.

† Root mean square deviations from ideal values. These refer to a dictionary of standard groups as specified in Table 2 of Sielecki *et al.*¹⁷.

‡ Recommended¹⁷ estimates of standard deviations which are used to determine the relative weights of restraints.

sporins react differently with their corresponding β -lactamases⁵, and this behaviour seems to carry over to the cephalosporin sulphone elastase inhibitors such as I. With a stable substituent at the 7-position instead of a relatively good leaving group, however, ring opening might yet be favoured, leading to novel rearrangements and reactions. The 7'- β substituents in the cephalosporin antibiotics are thought to form part of a mimic of the backbone of the D-alanyl-D-alanine-containing substrates of the enzymes of bacterial cell wall synthesis¹⁵. As such, these substituents can be quite long. In the elastase inhibitors, 7'- α substituents must be short, because they correspond in turn to the side chain of L-amino acid substrates, and must fit the shallow S1 specificity pocket of the enzyme. Given the structure of the complex, 7'- β substituents would not point into the pocket, and as expected, such isomers were weak or inactive when compared to their 7'- α analogues¹.

In conclusion, our elastase inhibition programme has produced a series of selective β -lactam inhibitors which provide a model system for the observation of β -lactam chemistry in action. This system has both significant similarities and differences with the more familiar antibiotic targets. Not surprisingly, conventional β -lactam antibiotics and their analogues do not inhibit elastase and conversely, the β -lactam elastase inhibitors do not act as antibiotics¹. We hope that by examination of a series of compounds in this class, we will be able to clarify and improve upon the beneficial effects of the β -lactams in therapeutic environments.

We thank J. Kelly for advice on soaking inhibitors, W. A. Hendrickson and J. L. Smith for discussions of the protein least-squares refinement program, E. Fluder for implementing the program on an IBM system, W. Steigemann for the use of the PROTEIN package, B. Bush and R. Blevins for graphics support, and S. Fischbach for help with the manuscript. The continuing support of our studies by E. H. Cordes is greatly appreciated.

Received 24 November 1986; accepted 20 March 1987.

1. Doherty, J. B. *et al.* *Nature* **322**, 192–194 (1986).

2. Janoff, A. A. *Rev. Med.* **36**, 207–216 (1985).

3. Stein, R. L., Trainor, D. A. & Wildonger, R. A. *Rep. med. Chem.* **20**, 237–246 (1985).

4. Brown, A. G. *J. antimicrob. Chemother.* **10**, 365–372 (1982).
5. Pratt, R. F. & Faraci, W. S. *J. Am. chem. Soc.* **108**, 5328–5333 (1986).
6. Knowles, J. R. *Acc. Chem. Res.* **18**, 97–104 (1985).
7. Boyd, D. B. *J. mednl. Chem.* **27**, 63–66 (1984).
8. Kraut, J. A. *Rev. Biochem.* **46**, 331–358 (1977).
9. Cohen, G. H., Silverton, E. W. & Davies, D. R. *J. molec. Biol.* **148**, 449–479 (1981).
10. Schechter, I. & Berger, A. *Biochem. biophys. Res. Commun.* **27**, 157–162 (1967).
11. James, M. N. G., Sielecki, A. R., Brayer, G. D., Delbaere, L. T. J. & Bauer, C.-A. *J. molec. Biol.* **144**, 43–88 (1980).
12. Cohen, N. C. *J. mednl. Chem.* **26**, 259–264 (1983).
13. Page, M. I. *Acc. Chem. Res.* **17**, 144–151 (1984).
14. Lamotte-Brasseur, J., Dive, G. & Ghysen, J.-M. *Eur. J. med. Chem.* **19**, 319–330 (1984).
15. Waxman, D. J. & Strominger, J. L. A. *Rev. Biochem.* **52**, 825–869 (1983).
16. Hendrickson, W. A. & Konnert, J. H. *Computing in Crystallography* (eds Diamond, R., Ramaseshan, S. & Venkatesan, K.) 13.01–13.23 (Indian Academy of Sciences, Bangalore, 1980).
17. Sielecki, A. R. *et al. J. molec. Biol.* **134**, 781–804 (1979).
18. Sawyer, L. *et al. J. molec. Biol.* **118**, 137–208 (1975).
19. McPherson, A. *Preparation and Analysis of Protein Crystals* (Wiley, New York, 1982).
20. Navia, M. A., Springer, J. P., Poe, M., Boger, J. & Hoogsteen, K. *J. biol. Chem.* **259**, 12714–12717 (1984).
21. Sparks, R. A. *Computational Crystallography* (ed. Sayre, D.) 1–18 (Clarendon Press, Oxford, 1982).
22. North, A. C. T., Phillips, D. C. & Mathews, F. S. *Acta crystallogr. A* **24**, 351–359 (1967).
23. Saper, M. thesis, Rice Univ., Houston (1983).
24. Steigemann, W. thesis, Technische Universität, München (1974).
25. Gund, P., Andose, J. D., Rhodes, J. B. & Smith, G. M. *Science* **208**, 1425–1431 (1980).
26. Jones, T. A. *Computational Crystallography* (ed. Sayre, D.) 303–317 (Clarendon Press, Oxford, 1982).
27. Bush, B. L. *Computers Chem.* **8**, 1–11 (1984).

Corrigendum

Evidence for carbonate in the mantle

G. W. Berg

Nature **324**, 50–51 (1986)

IN this letter reference should have been made to three examples^{1–3} rather than just a single instance¹ of minute inclusions of dolomite in samples of rock from the mantle ever having been reported despite 25 years of intensive study of these rocks. The author thanks Drs J. V. Smith and D. Smith for pointing out these omissions.

Erratum

A genetic pathway for the specification of the vulval cell lineages of *Caenorhabditis elegans*

Edwin L. Ferguson, Paul W. Sternberg
& Robert Horvitz

Nature **326**, 259–267 (1987).

IN Table 2 of this article, the lines referring to mutants *lin-17* and *lin-18* should appear beneath *lin-11* in the 'Expression mutants' section, not under 'Determination mutants'. Also in Table 2, a line referring to 'Cell fates' for the wild type was omitted, and it should read:

	Cell fates						P(1, 2, 9–11).p	No.
	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p		
Wild type	3°/S	3°	2°	1°	2°	3°	S	—

Finally, in Table 2 in the line referring to mutant *lin-12(0)*, the alleles used were *n137* and *n720* (the solidus, /, is misleading in the printed version).

Intelligent scientific documentation

Jeffrey M. Wales

Advances in computers permit scientific documentation systems to link with research data bases. Such 'intelligent' scientific documentation gives access to the data and computing resources behind the results.

INTEGRATING the rapidly increasing number of computing resources within modern research organizations has been viewed traditionally as a data communications problem. Accordingly, many companies have begun to make substantial investments in both local and wide-area networking. But without software applications that can actually take advantage of such communications capabilities, the initial promise of networking remains unfulfilled. The inability to provide functional access to scientific data to researchers throughout an organization can have a major influence on research productivity. To solve this problem, a new method for the production and communication of scientific documentation, including integrated text, graphics and the underlying research data, has been developed. It provides a network-based system for integrating document production research data bases throughout a distributed computing environment.

Compiling and communicating information among research groups and between laboratory facilities, as well as integrating information from the scientific literature and external data bases, are critical components of the research process. Unfortunately, these requirements are not ordinarily satisfied by the mere ability to transfer data files between one computer and another, or by sending an electronic mail message. To make the most effective use of the range of information and types of data included in the typical technical or scientific document, a user needs access to more than the document's textual or numeric data content. To make best use of a document's information content as a design or research tool, a user needs access to the underlying data files from which the document was derived, the software application programs that were used to process the data, and the computing resources on which those applications are hosted. To analyse or compare the information content of a document with related documents or research data (within or outside the researcher's own laboratory environment) it is desirable to gain access to the data bases that generated the document.

Conventional network software and desktop or technical documentation publishing systems do not address this problem directly. As a result, researchers either remain isolated from needed data, or must take a series of manual, time-consuming, and error-prone steps in order to obtain access to the data in the form required to do productive work. The lack

of an automated method means these steps must be repeated each and every time revisions are needed or variations of the problem arise. Often, performing these tasks correctly requires a considerable amount of expertise in the use of computers. Thus, available computing resources may be underutilized in reality, while the perceived need for additional equipment continues to grow. To solve this problem, a fundamental organizational framework for processing and analysing research data is required.

Modelling research

Conventional office automation or technical documentation systems are intended to produce images of documents for printing or other means of physical reproduction; they are not concerned with the information content of the document. The term 'intelligent scientific documentation' is intended to define computer-generated documents which can combine both text and graphics, including diagrams, pictures or charts, in such a way that the information content of the text and graphics is functionally accessible to the reader of the document. By allowing the reader to access the full information content of the page, whether to analyse or re-analyse the data, or to transfer the data to another software application, the technical document can become an active design tool rather than a passive storage medium.

The system can increase productivity within a research organization by providing researchers throughout the organization with the means to construct 'intelligent' scientific documentation. The sys-

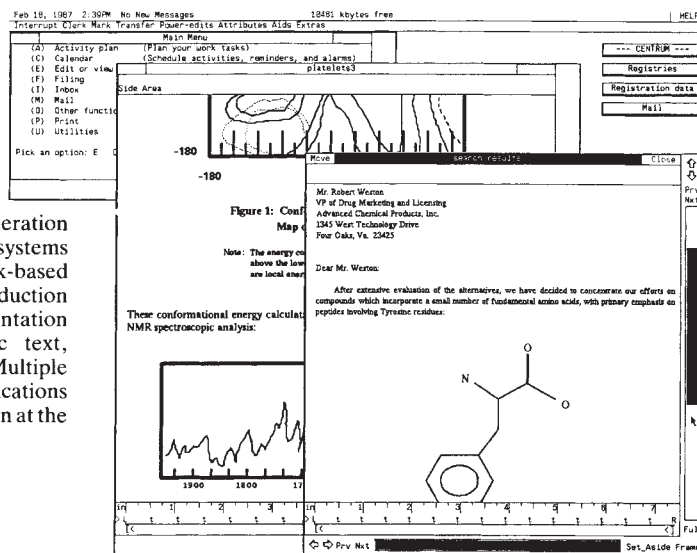


Fig. 1 The latest generation of documentation systems provides a network-based solution for the production of research documentation containing scientific text, graphics and data. Multiple documents and applications can be open on-screen at the same time.

tem can provide the ability to gain access to the data and application software that generated the document — all by interacting with the document itself.

By taking advantage of modern network facilities as well as Ethernet and TCP/IP communications standards, a system user can send and receive technical documents, complete with graphic content, within a department or across a company. Because the document contains logical pointers to the data and software applications used to construct it, the reader of the document can also obtain access to the data and the relevant software applications from within the document. This frees the user from the burden of dealing with unfamiliar computer operating system interfaces and file structures, and allows direct access to the information for more productive work.

The system is also designed to permit the integration of proprietary software applications and data types into the operating environment. Fully documented development tools can make the development of proprietary or third-party applications a practical reality.

Because the ability to distribute access to a system cost-effectively is a critical factor in achieving real productivity gains, the system is designed to operate on a range of conventional character terminals, IBM PC/ATs and compatibles, next-generation desktop workstations, and time-shared departmental computers with both UNIX and VAX/VMS operating systems. A consistent, menu-driven format and simple pointer operations ease training requirements and facilitate user

acceptance across a wide cross-section of the research organization.

Future of research automation

In the past, efforts to automate scientific research have concentrated on improving the quality, reliability, and speed of the experimental process. While there is no question that improvements in that area are (and will continue to be) needed, concentration on the experimental process has obscured to some extent the more basic need to deal more effectively with the increasing amounts of data produced by research. Without corresponding improvements in the researcher's ability to analyse, present and communicate the results of an experimental process in a way that can be shared by others, the value of the experimental data to the organization as a whole can be compromised.

To implement a research automation system effectively within a scientific setting, the organizational overhead associated with operating the system must be kept to a minimum. This requires that the majority of the data processing activity be

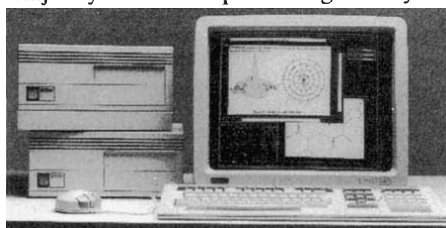


Fig. 2 The Centrum technical information management system, built around an advanced document composer, provides a unified computer-based research environment for scientific researchers, managers and support staff in the chemical and pharmaceutical research industry.

accomplished without the need to adopt unfamiliar procedures or learn an arcane command language. The scientific document was chosen as the principal metaphor for operating the system and organizing information and computer resources, because this is how technical professionals intuitively view their work. By adopting this type of object-oriented structure, it is possible to transform the data collection, analysis, and storage function from mere record-keeping activity into an active tool for design and discovery.

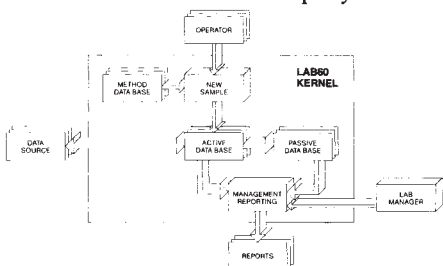
Polygen's recently announced Centrum system provides such an object-oriented approach to dealing with research data bases and technical information. While the initial application of the system will be directed almost certainly toward improving the quality and content of research documentation, over time its widespread use may effect fundamental improvements in the organization and use of computing resources. □

Jeffrey M. Wales is at Polygen Corporation, 200 Fifth Avenue, Waltham, Massachusetts 02154, USA. For additional information, fill in reader service number 100.

Laboratory software

From specialized wordprocessing to data crunching, laboratories have complex computer needs. Here is an example of software products on show at the Scientific Computing and Automation Conference, 13-15 May, in Amsterdam, the Netherlands.

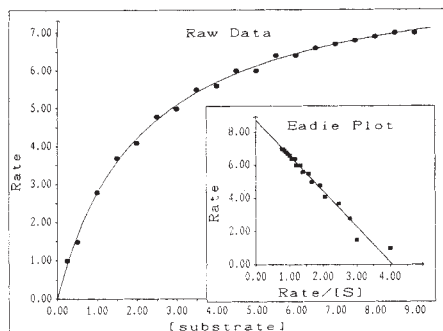
FROM Philips, in booth 22, comes a software package to provide **sample data management** for multi-instrument laboratories (Reader Service No. 101). Intended for production and quality control applications, the LAB60 software runs on the DEC PDP 11 family of computers. LAB60 may be used with any equipment capable of delivering suitable ASCII output, and allows results from online and offline sources to be merged into one analytical report. The DFL 50,000 software has what the company calls a



The flow chart for the LAB60 software shows its modular format.

"layered" structure: an active data base retains information on samples of current interest, while less urgent data intended for archiving are transferred to the second-level passive data base. Optional modules for reporting and statistics, quality search and charge correction are available.

Enzyme kinetics studies have now been simplified with the introduction of Elsevier-Biosoft's Enzfitter software (Reader Service No. 102). The \$140 (US) Enzfitter program, to be on display in booths 20 and 21, fits two sets of experimental data to one of several different equations, including: linear regression, pK_a determination, Michaelis-Menten kinetics, binding, single exponential, double exponential and first order rate equations. Extra sets of data and transformed/derivative plots of the same data



Enzfitter can display raw data and the results of kinetic analyses on the same screen.

can be shown on screen at the same time, and the results can then be printed in tabular or graphic form. The list of transformed/derivative plots includes residual, Scatchard, Eadie, Lineweaver-Burk, semi-logarithmic, and linearized pK_a formats. Enzfitter runs on 384K RAM IBM PCs with DOS 2.0 or later, and supports Hercules, Colour and Enhanced graphics cards.

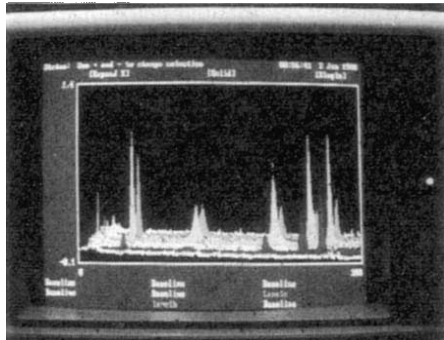
ChemText, by Molecular Design, is a software program that allows chemists to **integrate text with chemical structures**, reaction diagrams, equations and flow sheets to produce reports and documents (Reader Service No. 103). Molecules, reactions, forms and equations can be produced using ChemText's drawing editors, using boxes, lines, arcs, circles, arrows and a wide range of character types. Images from other sources may be placed in ChemText documents, including RS/1 graphic images and Molecular Design Metafiles. Files from other Molecular Design software, including those for molecular modelling, may also be imported to ChemText. ChemText has a \$1,500 (US) price tag, and will be demonstrated in booth 18A. It runs on IBM PCs and 100 per cent compatibles, and the Hewlett-Packard Vectra.

Biodata has two new **data acquisition packages** for waveform capture and data logging, to be displayed in booth number 24 (Reader Service No. 104). Biodata says the waveform capture package allows the user to capture data at speeds of up to 1 million samples per channel per second, and to recall, display and analyse the captured waveforms. The data logging package is designed for analogue inputs including thermocouples and RTDs, and can handle up to 256 inputs. Designed for use with Biodata's Microlink data acquisition system and IBM PC, XT, AT's or compatibles, the packages cost £695 (UK) each.

A program available from Perkin-Elmer allows the analysis of a differential scanning calorimeter (DSC) peak for the **kinetic parameters** that characterize a reaction process (Reader Service No. 105). Intended to analyse data obtained from the DSC 7 differential scanning calorimeter, a component of the £18,470 (UK) Perkin-Elmer 7 Series thermal analysis system, the software has various applications. It allows the analysis of exothermic or endothermic peaks, the use of

straight or sigmoidal baselines, and the generation of data in either graphic or tabular form. Kinetic parameters including heat of reaction, reaction order, pre-exponential constant, activation energy, and change in heat capacity due to the reaction can all be calculated with the program, to be demonstrated in booth 3.

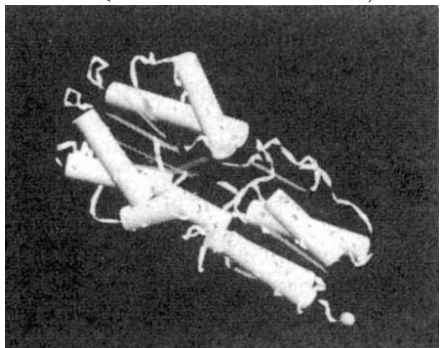
The Beckman DU Data Leader software for IBM PCs helps researchers draw conclusions from **UV-visible spectroscopy data** from the Beckman DU Series spectrophotometers (Reader Service No. 106). Using the program, spectroscopic data can be displayed on screen in various



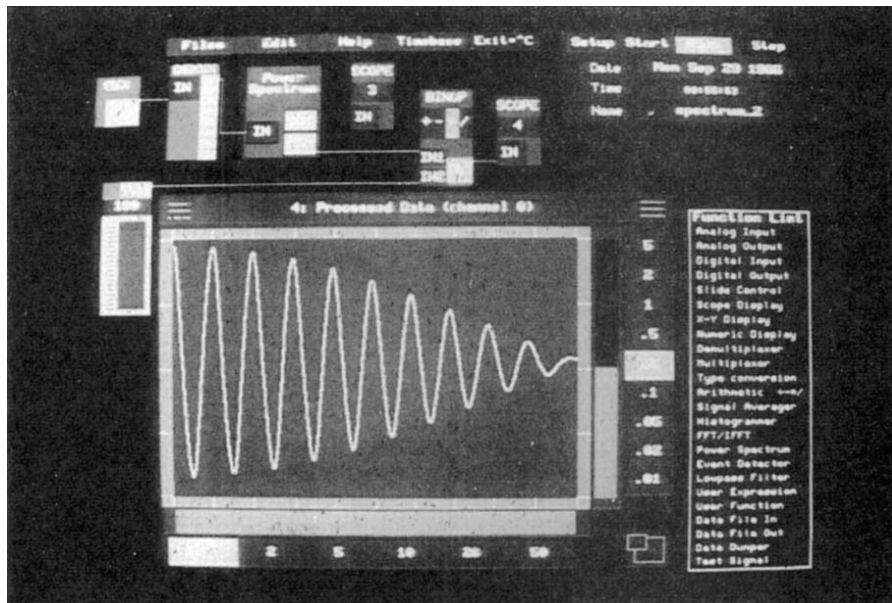
Beckman's Data Leader software is useful in the interpretation of spectrophotometric results.

graphical formats, and be manipulated mathematically with instant visualization of results. A hard copy of both can be printed out for presentation or report writing. Beckman says the pop-up help windows and the lack of the need to learn a programming language make the \$2,200 (US) Data Leader software easy to use. Audit trails, 'macros' that allow the consecutive use of various commands, and compatibility with Lotus 1-2-3 make Beckman's program especially functional. Data Leader can store a virtually unlimited number of data files, according to Beckman, which can be recalled at any time for comparison or manipulation. Booth 14 will have more information on Data Leader.

The latest molecular modelling software from Chemical Design is ChemProtein, a program designed especially for **protein engineering and pharmaceutical research** (Reader Service No. 107). Sure



In the ChemProtein model of the protein ABP, cylinders represent helices, and arrows sheets.



Data flow diagrams let Laboratory Workbench users set up for data acquisition without writing code.

to be demonstrated in booth 13, ChemProtein can display both traditional space-filling and stick models, and has a new, more natural, backbone ribboning display mode. Proteins may be built up by specifying amino acid sequences, or by reading in data from sources such as the Brookhaven Protein Data Bank. The £9,000 (UK) ChemProtein software can insert, delete or substitute amino acid residues while leaving key sites and secondary structures intact, a useful property for site-directed mutagenesis studies. Conformational analyses can also be carried out using ChemProtein's set of residue-dependent rules. ChemProtein is sold as a part of Chemical Design's Chem-X system, and runs on any DEC VAX computer with a VMS operating system. A discount of 90 per cent is available for academic users.

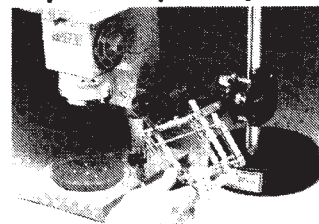
Masscomp has streamlined the process of setting up a computer to **acquire, process and display experimental data** in real time (Reader Service No. 108). With Masscomp's Laboratory Workbench software, researchers can move on to data acquisition without having to write their own programs, a process that often slows down an experiment. The mouse- and menu-driven software, to be demonstrated in booth 16, operates by data flow diagrams entered by the user. The \$3,000-4,900 (US) Laboratory Workbench is compatible with Masscomp's MC5000 microsupercomputers, and features pull-down menus, pop-up displays, graphical icons and interactive controls. Laboratory Workbench creates virtual instruments

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

out of spectrum analysers, oscilloscopes, high-speed transient devices and FM recorders, and can be used to design, test and run experiments. □

ADVERTISEMENTS

SINGER INSTRUMENTS Singer Instruments Xenopus oocytes injection?



SINGER MKI MICROMANIPULATOR

We also offer a Microsyringe Kit.

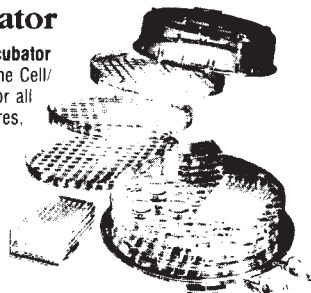
Write to:

Singer Instrument Co. Ltd.
Treborough Lodge
Roadwater, Watchet
Somerset TA23 0QL
England. Tel. (0984) 40226
Telex: 337492 Comcab G
Reader Service No.50

Versatile Tissue Culture Incubator

**Modular Incubator
Chamber** (the Cell/
Sphere™) for all
tissue cultures,
aerobic or
anaerobic.

**Low
Cost**



Eliminates evaporation loss and contamination. Easy to use - flush with gas mixture, seal, place at appropriate temperature. Stackable, see-through. Quantity discounts. billups-rothenberg, inc., pob 977, Del Mar, CA USA 92014. Call 619-755-3309 or contact your Flow Laboratories representative.

Reader Service No.6

Researchers living on the fringe

Richard Pearson

The academic world in Britain is changing rapidly due to government action and economic pressures.

THE British government's long-awaited White Paper on higher education has now appeared, offering a package of reformed structures, vocationalism, and increased student numbers, all within a broadly static budget¹. The key to the future is clearly going to be market forces, with the labour market having a far greater role in determining course balance and structures, and, it is hoped, student choice. But in what promises to be the most dramatic change, a competitive market is going to be set up between institutions as they seek to 'win' the most favourable contracts from the national funding bodies. For — if returned in the forthcoming general election — the government plans to reform or indeed replace the existing funding and 'arms-length' management bodies of the University Grants Committee and the National Advisory Board by funding agencies which will place contracts with higher education institutions to deliver different kinds of services, be they the education of different types of graduate, the carrying out of research, or the provision of continuing education. At the same time higher education institutions will be encouraged to enter into more direct contracts with industry for research, consultancy and technology transfer.

The net effect is likely to be much greater diversity between institutions, as success breeds more success and expansion for a few, others will increasingly specialize and seek out their market niche, while others will contract and decay. Whether the full logic of the market will follow through, allowing some to close and indeed new institutions to be born, American-style remains to be seen. What is clear is that new employment relationships will emerge as higher education institutions have to manage greater uncertainty as to the size and balance of their workloads and become more flexible in setting and maintaining employment levels. Tenure will become increasingly rare and short-term and part-time contracts more prevalent.

This has already happened in many areas of industry and commerce where increasing reliance is being placed on a 'periphery' of temporary, short-term contract staff, part-timers, and freelancers and the self-employed as firms cope both with fluctuating work-loads and changing skill needs, while a 'core' of stable employment is maintained. For the majority in this core who will have to work more flexibly, then

employment security is usually high and training and career development is more often available. This increasing practice, dubbed the 'flexible firm', does, however, depend in part on there being an adequate supply of under-utilized skills in the wider labour market. Where this is the case the people in the periphery normally have poorer working conditions and receive little or no training to help them keep their skills up to date and be able to do the jobs of tomorrow. There are some groups who can, however, take advantage of this status, where, for example, there are skill shortages and they can sell their time at a much higher rate as a freelancer than as a salaried employee. In the universities and polytechnics a similar phenomenon has applied for many years, with a core of tenured lecturers and a periphery of contract researchers.

In the case of the universities, a study for the Advisory Board to the Research Councils just out² shows that short-term contract researchers are increasingly being used by research councils, industry, foundations, government and overseas sponsors to fulfill their research needs, with the numbers rising by over 70 per cent in ten years to total of 10,000 in 1985. This was during a period when the number of university-funded academic staff fell by 5 per cent to just over 31,000. In 1984 about half the contract researchers were funded by the research councils who had a total budget of over £540 million, with the Science and Engineering Research Council paying for about 3,000 and the Medical Research Council (MRC) nearly 1,200. These two funding bodies increased not only their numbers over the period but also their share of contract researchers (Fig. 1). This concurs with the shift in the research councils' budget away from expensive capital towards people in

intensive areas such as engineering. On a subject basis, medicine and engineering both increased their share, science kept its proportion, while that for all others fell. On a cost-centre basis, clinical medicine accounted for one in six researchers, with major financial support coming from a wide range of sources including the MRC, government, foundations and industry.

The researchers themselves were still predominantly male, although women

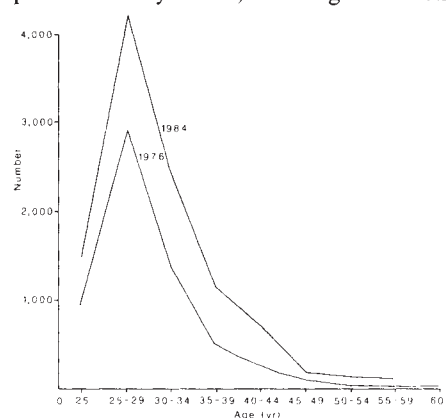


Fig. 2 Age distribution of short-term contract researchers.

were an increasing proportion, totalling over 28 per cent in 1984. One in four researchers had a PhD, while all but a few of the remainder had first degrees. As a group they are ageing, with 42 per cent over 30 in 1984, a proportion that had risen from 35 per cent 8 years earlier. The main age cluster, however, remained in the range 25–29 (40 per cent, Fig. 2). As well as ageing, the length of stay in contract research is also lengthening, with 40 per cent having been in their fourth year or more in 1984. There is still a large flow of people through these posts, with one in four leaving after one year.

These statistics provide only a partial picture of the pool of contract researchers, a group which is likely to expand further in the future and to be complemented by an additional group of contract teachers. For the present it is likely that there will be a pool of candidates willing to chance their luck with these jobs, perhaps because they see them as the springboard into more permanent academic careers. However, if previously permanent career posts are transformed into itinerant posts the supply of candidates may start to dry up. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Falmer, Brighton BN1 9RF, UK

1. Johnstone, B. *Nature* 326, 531 (1987).
2. Varlaam, C. *Contract Researchers in Universities* (IMS/ABRC, 1987).

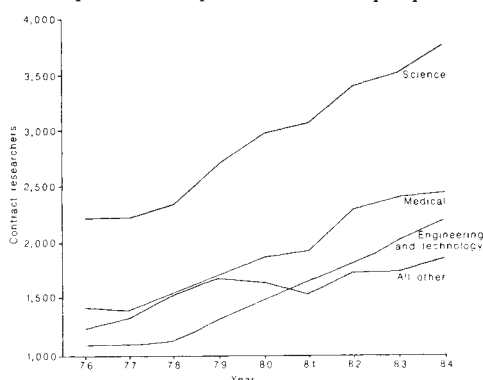


Fig. 1 UK contract researchers by subject group, 1976–84.